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STUDIES OF THE FORMATION AND TRANSLOCATION OF CARBOHYDRATES IN PLANTS.

I. THE CARBOHYDRATES OF THE MANGOLD LEAF.

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INTRODUCTION.

THE object of the investigations recorded in the present series of papers was to throw light on fundamental problems—how carbohydrates are formed in the foliage leaves of plants, how they are transferred to the reservoirs where they are stored (as saccharose in the sugar beet or mangold[1], as starch in the potato and the cereal crops and as inulin in many Compositæ such as the artichoke or dahlia) and how they are finally broken down and utilised in subsequent growth. A complete account of the work done in this field up to the year 1893 was given by Brown and Morris [1893] in their classical paper on "The Chemistry and Physiology of Foliage Leaves."

Sachs [1862] first proved that the production of starch in the chlorophyll granule depends on the action of light and that the starch formed during the hours of sunlight is wholly or partially redissolved and removed from the leaf during the night to supply the demands of the growing points of the plant. Sachs regarded the starch as the "first visible product of assimilation" and considered that *all* the carbohydrate synthesised in the leaf passed through the starch stage; he was of opinion that the starch disappeared in the form of sugar. Schimper [1885] on the other hand held that starch is not only converted into sugar in the plant but, from his observations of the increase of starch in leaves supplied artificially with solutions of sugar, concluded

[1] Both the sugar beet and the mangold are varieties of *Beta vulgaris* L. and apparently have been derived by cultivation from the *Beta maritima* of our coasts.

that glucose precedes starch in the process of assimilation, the starch being formed from the glucose when the concentration of the latter exceeds a certain maximum which differs in different plants. Taking into account Baeyer's hypothesis, advanced in 1870, it was possible to regard the glucose as formed by the polymerisation of formaldehyde, CH_2O, which was itself produced directly by the reduction of carbonic acid in the leaf under the influence of light and chlorophyll.

Some such view as this appears to have been generally held down to 1893, although Arthur Meyer [1885] emphasised the fact that the formation of starch in plants was by no means universal[1]; many monocotyledons, particularly the *Liliaceæ*, form very little starch in their leaves, some none at all (e.g. *Allium cepa*, *Scilla maritima*). An important point established by Meyer was that leaves of plants which store starch abundantly contain comparatively little sugar, whilst plants like *Gentiana lutea*, *Allium* and Asphodel, which form little or no starch in the leaf, accumulate large stores of soluble reducing substances, probably glucose. When starch is absent Meyer considers that its place may be taken as a reserve substance by other sparingly soluble carbohydrates, such as inulin (*sinistrin*), which he isolated from the leaves of the Yucca. In 1886 Meyer [1886] made the interesting observation that almost all leaves which are capable of forming starch at all, produce it abundantly from a 10 per cent. solution of laevulose and a relatively small number from dextrose.

The important work of Brown and Morris in 1893 may be regarded as marking the beginning of a new period in the study of the physiology of the leaf. Up to this date, the chemical methods used had been largely qualitative or very roughly quantitative; little attempt had been made to discriminate between the reducing sugars of the leaf, which were universally regarded as glucose (dextrose). The possibility of laevulose (fructose) being present or a mixture of laevulose and dextrose (invert sugar) had hardly been raised, whilst maltose had not been suggested as a possible leaf carbohydrate, although it was known that this sugar was formed from starch by the action of diastatic enzymes. Cane sugar, although known to be widely present, had not been taken into account as a possible direct product of photosynthesis (except by A. Girard [1884] in an important paper referred to later); it was generally regarded as a reserve carbohydrate formed as a secondary product from the simpler sugars.

[1] Boehm [1856] had found that the chloroplasts of *Allium* species, *Galanthus*, *Hyacinthus*, *Ornithogalum* and *Iris germanica* normally do not form starch.

Brown and Morris from their study of the *Tropæolum* leaf were able to bring forward much evidence for the rejection of Sachs' view that all the carbohydrate formed in photosynthesis passes through the starch stage. The facts they adduce are more in accord with the idea put forward by Meyer in 1886 that starch is only produced when the supply of formative carbohydrates is in excess of the metabolic and translocating powers of the cell in which they are contained. Starch begins to be stored in the leaf when the concentration becomes too great for the normal requirements of the cell; the separation of the sugar as starch, that is in an insoluble form, relieves this concentration. The subsequent dissolution of the starch at night was shown by Brown and Morris to be brought about by an enzyme similar in its nature to the diastase of barley. All the plant leaves tested which form starch were shown to contain such an enzyme. Brown and Morris proved, moreover, that leaf diastase acts upon solid starch and that the amount of diastase in the leaves increases with the time that the leaf has been in darkness; this is not due to the fact that it accumulates as the starch disappears, owing to a diminished call upon it, but probably on account of diastase being produced as a starvation phenomenon. In accordance with the view that the starch is broken down by diastase, they give analyses showing the presence of maltose in oven-dried *Tropæolum* leaf. From similar material they isolated an osazone which, from an analysis and melting point, appeared to be identical with maltosazone. Maltose was regarded as a normal constituent of foliage leaves formed as a degradation product from the starch.

One of the most novel conclusions drawn by Brown and Morris was with regard to cane sugar. This was found to be present in even larger proportions than starch and the way in which it fluctuated in the leaf suggested that it was the first sugar formed in photosynthesis. Dextrose and laevulose were also present and were more readily accounted for as products of the hydrolysis of the cane sugar than as its precursors. Since laevulose was generally in excess of dextrose it was suggested that the latter is more quickly used up in respiration than the laevulose; therefore it seemed probable that under natural conditions a larger amount of laevulose than of dextrose passes out of the leaf into the stem in a given time.

In 1884 A. Girard[1] [1884] had also suggested that the saccharose

[1] Girard gives a valuable summary of the investigations prior to 1884, dealing with the special problem of the formation and transmission of sugar in the sugar beet. In 1883 Kayser [1883] had found, by analysis, both cane sugar and reducing sugar in the

which is stored in the root of the sugar beet is formed in the leaf of the plant by direct photosynthesis and transferred as such to the root. Girard's work, which from the quantitative standpoint was far more complete than anything previously published, was apparently overlooked by Brown and Morris in their 1893 paper. Girard's views were based on numerous analyses which showed the roots to contain saccharose only and no reducing sugar; reducing sugars, however, were present in the stalks (petioles) and leaf tissue. The roots and petioles were found to have the same composition by night as by day, but the proportion of saccharose in the leaves was much greater—frequently twice as great—at the end of a day's insolation than next morning, after being several hours in darkness. The proportion of reducing sugars in the leaves, however, was sensibly the same in the evening as next morning and only increased as the plant developed. We shall discuss Girard's data more in detail later.

Since 1893 several papers have been published in which the formation of carbohydrates in the leaf is considered. These may be divided into two classes: (1) Those whose authors favour the view that saccharose is the first sugar formed in photosynthesis, (2) those in which the hexoses are regarded as primary products, the saccharose as formed later by synthesis either in the leaf or the root. We will briefly review these two classes separately.

1. *Cane sugar held to be formed directly.*

Went [1898] published observations on the distribution of reducing sugars and saccharose in the unripe sugar cane. The value of these is marred by the fact that, in the polarimetric estimation of cane sugar, Went calculated the reducing sugars as dextrose. Although Went held that cane sugar is the first formed sugar, he gives no experimental evidence in favour of this view. Strohmer [1908] relied upon the fact which was supposed to be established, first by Péligot and later by Girard [1884], that the roots of the sugar beet contain no sugar but saccharose; he states that, in the beet, reducing sugars never occur in the root, even in the early stages of growth. This, together with Girard's observation that in the night the saccharose content of the leaf fell to

leaves of several plants, e.g. *Beta vulgaris*, the grape vine (*Vitis vinifera*), potato, onion. In the early stages of growth, Kayser's analyses show that the cane sugar in the leaves is often greatly in excess of the hexoses, but later the proportion is increased. Kayser actually separated cane sugar from the leaves of the vine in a crystalline condition and with $[\alpha]_D = 62{\cdot}9°$.

one-half, is held to prove that the saccharose is formed in the leaf directly and migrates as such to the root in the night. He supports this view by observations on two abnormally formed beets in which the length of the neck had become enormously exaggerated; these abnormal growths contained cane sugar but no reducing sugar. If reducing sugars wandered from the leaf to the root they should have been found in large amount in the abnormally long necks. The objection to the view that the saccharose wanders as such, which is based on the supposed impermeability of the cell protoplasm to cane sugar, is, according to Strohmer, obviated if one assumes with Pfeffer that the protoplasm can change its properties periodically under the influence of a regulating mechanism. In a later paper [1911] Strohmer gives data for the second season's development of the sugar beet grown for seed, and shows that the total saccharose content of the root and principal stem is at the ripening period considerably greater than at flowering; the saccharose, however, during ripening entirely disappears from the leaves and stalks. This is held to confirm the view that the cane sugar is formed in the leaf and leaves it as such. Reducing sugars, however, predominate in the parts of the plant above ground at the flowering period because they have been formed by the inversion of saccharose, so as to be readily available for the building up of the flowers. Thus, for example, in the main stalk the ratio of reducing sugars to saccharose was 11·2 : 4·3. But later on, when ripening is near, the reducing sugar disappears almost completely.

Stephani [1911] also holds that saccharose is formed in the leaf of the beet and is transferred as such to the root for storage; the proportion of the reducing sugars in the root is generally very small (0·05 to 0·10 per cent.), but in some feeding varieties (*Futterrüben*) may be as high as 0·5 per cent.

Parkin [1912] made a careful study of the sugars present in the leaf of the snowdrop (*Galanthus nivalis*), to which we shall refer more in detail later. He considers that his analyses strongly favour the view that saccharose is the first recognisable sugar to be formed in the leaf and that the hexoses arise from it through inversion. The rapid rise in cane sugar, which occurs in the leaf when it has been exposed to darkness for some days and is then again placed in sunshine, is particularly striking; thus in one experiment the cane sugar rose after 8 hours exposure to sunlight from 5 per cent. to 12·5 per cent., whilst the hexose changed only from 2·7 to 3·6 per cent. In darkness, on the other hand, it is the saccharose which falls rapidly whilst the hexoses remain nearly

constant. Parkin's experiments were carried out with a plant, the snowdrop, which in normal growth never elaborates starch, so that complications which might arise from the presence of this carbohydrate or of maltose (which was shown to be absent throughout) were avoided. Parkin's view, which was also put forward by Brown and Morris in 1893, that the laevulose is present in excess of the glucose in the leaf—pointing to the latter contributing more readily to the needs of the leaf—is discussed in the next paper.

Peklo [1908], who studied the localisation of sugars in the beet by a microchemical method, concluded that the sieve-tubes of the phloem contain the greatest amount of cane sugar; he considers that the sieve-tubes serve mainly for the transit of the sugar and, after the formation of callus plates, for the storage of sugar in the root.

2. *Reducing sugars (hexoses) held to be the primary products and cane sugar to be formed from these.*

Maquenne [1895], in an attempt to explain the storage of saccharose in the beet, based on osmotic laws, stated that the osmotic pressure of the leaf sap is practically identical with that of the root sap. As it is essential to equilibrium that the concentration of the saccharose should be double that of the invert sugar, when, owing to photosynthesis, reducing sugars are formed in the leaf, they will travel to the root and there take up the form of saccharose.

Strakosch [1907] employing microchemical methods concluded that dextrose is formed in the mesophyll of the leaf of *Beta vulgaris* and is the only sugar found therein. The migration of dextrose into the leaf veins is followed by the appearance of laevulose in these, and later by the formation of saccharose. Strakosch considered that the cane sugar must be regarded as a final product in the leaf and migrates to the root as such. The amount of the monosaccharides in the leaf is not appreciably altered by the migration of the saccharose to the root, nor is it diminished when the leaves remain in the dark for some time. Exposure of the leaves to light does not cause the saccharose to increase beyond a certain maximum, which is attained in a short time.

In 1909 Robertson, Irvine and Dobson [1909] studied the distribution of enzymes in the roots, stalks and leaves of *Beta vulgaris*; they showed that invertase is present in the leaf and stem but absent from the root, and hence conclude that the cane sugar stored in the root is formed from antecedent monosaccharides by reversible zymohydrolysis in the leaf and stem and is thence translocated as such. The

alternative view that the sugars travel downwards as hexoses, although meeting the diffusion difficulty, is not in accord with the absence of invertase in the root. Strohmer's analyses, which showed that practically no reducing sugars occur in the root, and Girard's conclusion that saccharose is present in all parts of the plant in the earliest stages of growth, also militate against this view.

Gutzeit [1911], on theoretical grounds based on the laws of diffusion and osmotic pressure, contended that monosaccharides are formed in the leaf and wander as such to the root where they are built up to cane sugar.

In the same year Ruhland [1911] contended that the sugar in the sugar beet does not pass from the leaf in the form of saccharose but as reducing sugars (perhaps largely as laevulose); the sugars entering the root consist mainly of reducing sugars which are resynthesised to saccharose within the root. The cells of the leaves and stalks are stated to be permeable to saccharose, raffinose, maltose and more or less to all the hexoses tested, from which they are able to form starch. Dextrose and laevulose are somewhat more diffusible than saccharose. Light does not appear to exercise any influence on the permeability of the cells, but on the other hand certain regulatory influences could be detected. Ruhland states that the young growing root contains invertase which gradually diminishes in quantity as the root matures and is confined only to the growing parts.

Deleano [1912], in a study of the respiration of the vine leaf (*Vitis vinifera*), found that only a small increase of reducing power was caused by inverting a solution obtained from the dried leaf material; this pointed to the presence of very small quantities of cane sugar relatively to the reducing sugars, which were present in large amounts, and as he failed to isolate any cane sugar by Schulze's strontia method he concluded that this sugar was in reality not present. We shall specially deal with this point later.

As distinct from the foregoing workers, who, on the one hand, regard saccharose as the first sugar formed in the leaf, and, on the other, consider the first product to be dextrose, Pellet [1913] considers that saccharose, dextrose and laevulose are formed simultaneously in the leaf and that the sugars descend in all three forms to the root, where the reducing sugars are built up to saccharose. In a later paper Pellet [1914, 1] shows that the view generally held, that the sugar cane contains no reducing sugars, is based upon early work which was carried out prior to the introduction of satisfactory methods of detecting or estimating reducing

sugars. According to Pellet the reducing sugars which are found in the juice of the crushed cane are present as such within the cane itself and are not formed by inversion of the expressed juice. The proportion of reducing sugar to cane sugar increases on passing from the lower to the upper parts of the cane—that is nearer to the leaves; it grows less and less as ripening proceeds, pointing to a conversion of the reducing sugars into saccharose. Colin [1914] takes a similar view to Pellet. He shows that Girard's and Strohmer's assertions that reducing sugars are absent from the root of the beet are quite incorrect, especially in the early stages of growth, when the reducing sugar may form 20 per cent. of the total sugars. As the root develops and the store of cane sugar increases in it, the proportion of reducing sugar naturally falls, but it never entirely disappears and is always most abundant in the growing parts. In the neck the ratio of reducing sugar to cane sugar is highest. The root therefore receives at the same time both cane sugar and reducing sugars: the former is stored up and the latter polymerised to cane sugar. The entry of the sugars is regulated by the osmotic pressure of the mixture.

The recent work of Campbell [1911] and Kluyver [1914] will be dealt with later.

Experimental.

Methods of Work.

Destruction of enzymes. Much of the earlier work on the carbohydrates of the leaf is of doubtful value because insufficient care was taken to ensure that no change in the carbohydrates should occur after the picking of the leaves and during the preparation of the sample for analysis; changes brought about by enzymes are, as we shall show, very liable to occur either during the process of drying the leaf or during the expression of the sap, if a press be used. Whilst it is possible to express the juice from the root of the beet or mangold, or from the sugar cane, without much change in the cane sugar occurring, owing to enzymes being almost entirely absent in such cases, with leaf tissue such a process is unsafe, as enzymes are present in relatively large amount and are liberated from the cells in which they are contained by the mechanical pressure and thus have an opportunity to act upon the sugars during the time taken in preparing the sample. The work of Girard [1884] in particular, although he states that he worked as rapidly as possible, was liable to error from this cause; the leaves of

the sample were first pulped in a special machine and then subjected to pressure so as to obtain the sap. Brown and Morris [1893], working with *Tropæolum* leaves, found in their preliminary experiments that considerable changes may occur in the sugars when the sample is prepared in this way, and Kluyver [1914] states that on estimating the cane sugar and reducing sugars in the sap of the same leaf he found practically no cane sugar, unless the juice were heated to 100° to destroy the enzymes before making the analysis. Brown and Morris in 1893, and Parkin [1912], and Kluyver more recently, therefore always attempted to destroy the enzymes in the leaves by quickly drying these in a steam oven. Parkin gives analyses which would indicate that in the case of the snowdrop this method of working brings about little change in the proportion of the sugars in the leaf. On the other hand, when the leaves are moderately thick, as in the mangold or sugar beet, and therefore heat up slowly, an opportunity is given to the enzymes to bring about considerable change in the carbohydrates before they are actually destroyed; enzyme action is especially likely to occur under these conditions, because the rate of action is greatly accelerated at first by the rising temperature. We shall show (see p. 357) that certain fundamental differences between our results and those of Brown and Morris and of Kluyver (for example, the entire absence of maltose in all the cases we have studied and our higher values for starch in the case of the *Tropæolum* leaf) are probably to be explained by enzyme action having taken place in the earlier experiments.

In all investigations hitherto, the material which was analysed was either pressed-out sap or an extract of the previously dried tissue. To ensure the destruction of the enzymes being as instantaneous as possible, we have adopted the following procedure. The freshly picked leaf material (about 1 kilogram) was dropped in small quantities at a time into a large volume (2 litres) of boiling alcohol, to which 1 per cent. by volume (20 cc.) of 0·880 ammonia was added so as to neutralise the acids present in the leaf; the quantity given is generally sufficient for this purpose. After the extraction with alcohol is complete, the solution is generally faintly alkaline to litmus paper; if this is not the case a little more ammonia should be added. By the method given it would appear that the enzymes in the plant tissue are instantly destroyed; the ammonia facilitates the destruction owing to its alkaline nature and its rapid diffusion into the plant cells. The nature of the results we have obtained gives us confidence that no changes occur in the leaf carbohydrates during this treatment or during the subsequent

extraction of the sugars. Had any change, such as inversion, occurred, it would have been impossible to obtain, for example, the regular series of results actually found in the case of the potato leaf (see p. 367), where the cane sugar rises and falls between very narrow limits (2·1 to 3·5 per cent.) along curves which are practically straight lines.

Methods of Analysis. If work such as we have been engaged on is to have any permanent value, it is necessary to ensure that the analytical methods give trustworthy results with the class of material actually dealt with. Much time was therefore devoted in the beginning to testing these methods, and we have shown in several earlier papers (Davis and Daish [1913 and 1914]) that very grave errors may arise in investigations of this kind. Thus the important work of Brown and Morris in 1893, so far as it refers to the sugars, suffers from the fact that the only method then available to estimate maltose gave entirely incorrect results owing to the destruction of laevulose brought about by the hydrochloric acid used in the hydrolysis; as the accuracy of the values for the other reducing sugars, dextrose and laevulose, depends upon correct values being taken for the maltose, it is clear that the proportions found for these sugars are equally incorrect. We have shown also (Davis and Daish [1914]) that, in estimating starch by the diastase method, large errors may be caused by the loss of dextrin; we have introduced therefore a new method based on the use of taka-diastase, the enzyme of *Aspergillus oryzae*, which converts starch into a mixture of maltose and dextrose only. The method of hydrolysing cane sugar which was used by Campbell [1911] may, we have shown, give rise to entirely false results when applied to plant extracts, owing to the cane sugar being only partly inverted by the 2 per cent. citric acid which suffices to invert completely *pure* solutions of the sugar. In Campbell's analyses the cane sugar would therefore be underestimated; this would lead to very high values being returned for maltose, even though considerable destruction of laevulose occurred during the hydrolysis with hydrochloric acid. As the values for saccharose and maltose were incorrect, the data for dextrose and laevulose are equally invalid. Campbell's work on the carbohydrates of the mangold leaf must therefore be regarded as merely preliminary in a very difficult field and the data and conclusions entirely withdrawn.

Parkin's recent work [1912] was, fortunately, carried out with a plant in the leaves of which starch and maltose do not occur. The analyses were therefore not complicated by the necessity of estimating these substances. Parkin carefully tested many points of the analytical

procedure used in estimating the cane sugar and reducing sugars; as will be seen later, many of our results confirm those obtained by Parkin (except as regards the dextrose : lacvulose ratio, a subject which is dealt with separately, see Paper II), so that considerable confidence may be placed in these observations. The principal points open to criticism are: (1) How far the results were affected by the process of drying the leaves adopted by Parkin; (2) how far the cane sugar results were lowered owing to incomplete inversion occurring on account of the presence of lead acetate in the solution interfering with the ordinary Clerget process. From the experiments Parkin actually made to test these points it would appear that in the case of the snowdrop the error arising from either cause was but small.

In earlier papers (Davis and Daish [1913 and 1914]) we have given an outline of the methods of analysis we have adopted; we need therefore only add a few details which were formerly omitted.

Cane sugar has always been estimated by two distinct methods: by inversion with 10 per cent. citric acid [1913, p. 466] and by inversion with invertase (autolysed yeast). This gives a means of checking the results.

Maltose was estimated by the use of maltase-free yeasts [1913, p. 464], such as *S. marxianus* and *S. exiguus*, duplicate fermentations being carried out with ordinary baker's or distiller's yeast so as to make allowance for the *pentoses* present which remain unfermented.

Starch was estimated by taka-diastase [1914, p. 159] in the dry leaf material from which the sugars had been completely extracted by alcohol. Special details are given of this method, as applied to estimate "soluble starch" or "dextrin" when present, in our experiments on the potato leaf (see p. 361).

Pentoses. As shown in a previous paper (Davis and Sawyer [1914]) appreciable quantities of pentoses are invariably present in the alcoholic extracts of leaf material; these have been estimated by distilling a known volume (50 cc.) of the original purified solution used in the sugar estimations with hydrochloric acid and weighing the furfural produced as phloroglucide according to the Kröber-Tollens method.

Pentosans. A suitable quantity (1·5 grm. of the oven-dried leaf material, from which the sugars have been extracted, or about 1 grm. of extracted stalk) is distilled with hydrochloric acid under Kröber-Tollens conditions (Allen's *Commercial Organic Analysis*, I. 402); the furfural evolved is precipitated and weighed as phloroglucide.

For actual examples of the method of calculation see *Appendix*, pp. 315 to 319. All results are calculated as a percentage on the *total vacuum-dried matter* of the leaf (T.V.D.M.), that is on the sum of the alcohol-soluble and alcohol-insoluble substances.

Extraction of the sugars from the leaf material and preparation of the solution for analysis. The freshly plucked leaf material (about 1 kilogram) was cut off close at the end of the stalk and, after cutting out the mid-ribs, was dropped, in small quantities at a time, into two litres of boiling 95 per cent. alcohol to which 20 cc. of 0·880 ammonia had been added, contained in a large zinc beaker (14 ins. × 9 ins.) in which the alcohol could be safely boiled; after each addition, the whole mass was well stirred so as to immerse the newly added leaves and ensure the rapid destruction of the enzymes. The whole kilogram of leaf could be added in less than 10 minutes; the time of picking each sample was also about 10 minutes, the picking being commenced 5 minutes before the hour and completed 5 minutes after. As the Laboratory was near at hand, the whole mass of leaf could be added to the boiling alcohol in less than 30 minutes from the nominal time of picking. The stalks and mid-ribs were separately dropped into a smaller quantity of boiling alcohol (1 litre containing 10 cc. of 0·880 ammonia) contained in a smaller zinc vessel (12 ins. × 5 ins.). After the leaf or stalk material had been added to the alcohol, the latter was kept boiling about half-an-hour; the alcohol was then drawn off and transferred to the boiling vessel *B* of a large specially constructed zinc extraction apparatus, shown in Fig. 1[1]. The leaf material was packed into the extraction vessel *A*, which was fitted with a detachable false bottom of perforated zinc and acted on the principle of the ordinary Soxhlet extractor, the alcohol siphoning back from *A* into the boiling vessel *B* during the extraction. *B* was heated by a large water-bath. Two condensers were fitted at the top of the extractor to condense the alcohol vapour which was conveyed from the boiling vessel by a bent tube of compo-metal so arranged that it could be easily fitted to or disconnected from the apparatus, through the corks of which it passed. This tube and the extractor itself were wrapped in felt to minimise air-cooling. The extraction was generally complete after about 12–18 hours; preliminary experiments showed that the whole of the sugars are removed when the leaf becomes colourless. To hasten the extraction, the leaves should be turned out of the extractor after about 10 hours

[1] For the mid-ribs and stalks a smaller extraction apparatus of zinc was used. The dimensions of vessel *A* were 7″ × 4″, and of *B* 5″ × 5″, with a 2″ neck.

and the portions which were at first at the bottom (and therefore less completely extracted and still green in colour) put back at the top, where the alcohol is hotter and acts more efficiently. In carrying out a series of pickings every 2 hours over a period of 24 hours, we have used four large extractors, the later samples being put into the apparatus

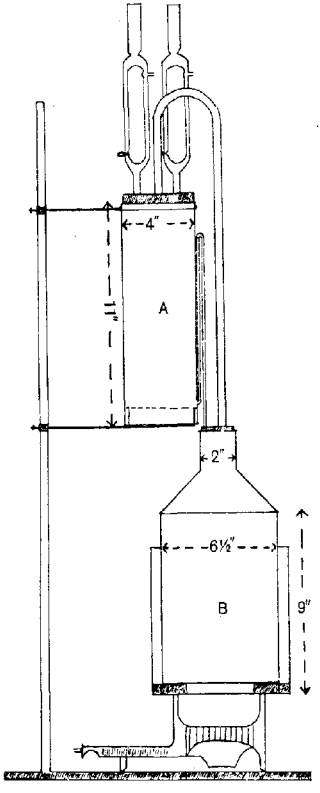

Fig. 1. Extraction apparatus. $\frac{1}{8}$ size.

as soon as the earlier samples were completely extracted. Three smaller apparatus served to deal with stalks and mid-ribs.

When extraction is complete (as judged by the leaves being practically colourless) the alcohol in the extractor is allowed to siphon over into *B* as completely as possible and the leaves are transferred to a small

jute bag[1]; the alcohol is then expressed by means of a Buchner hydraulic press. It is generally quite colourless and is added to the extract in *B*. The leaf material remaining is obtained, after the pressing, in a practically dry condition as a hard cake; it is shredded apart and the loose material obtained dried on paper trays in a steam oven for 18 hours; the mass obtained is then weighed on a rough balance (to the nearest centigram), quickly ground in a small mill, and left in an air-tight bottle until it can be analysed. In this material the *moisture* lost by drying at 110° *in vacuo*, the *starch* and *pentosans* are subsequently determined.

The alcoholic extract, which with washings, amounts usually to nearly 3 litres, if it cannot be immediately analysed (as is usual in a series of extractions) is transferred to a large bottle, about 10–20 cc. of toluene is added and the bottle closed with a paraffin-waxed cork. We have found, in preliminary experiments, that alcoholic extracts made in this way can be stored for 3 to 6 months without the slightest change, even inversion of the cane sugar, occurring. Care should be taken that the solutions are practically neutral or only *very* faintly alkaline to litmus paper; this is usually the case when ammonia has been added in the proportion stated above, but if any acidity can be detected in the solution it should be corrected by adding the proper quantity of ammonia so as to make the solution *just* alkaline to litmus.

The alcoholic extract serves to estimate the *total soluble matter* and the *sugars* of the leaf (saccharose, maltose, dextrose, laevulose and pentoses). For this purpose it is evaporated *in vacuo* (20–30 mm.) in the special apparatus devised for this work (Davis [1913]) which needs practically no attention and enables the 3 litres of extract to be reduced to a small volume (100–150 cc.) in a few hours at a temperature not exceeding 35–40°. At so low a temperature all possibility of change in the sugars is obviated. When the extract has been reduced to 150 cc. it is transferred to a 500 cc. measuring flask[2]. When much

[1] This should always be boiled with water several times before use to extract any soluble substances (dextrin, etc.).

[2] In the case of stalks and mid-ribs the alcoholic extract (usually about 1500 cc.) is evaporated *in vacuo* to about 25–30 cc.; it is then transferred, with washings, to a 100 cc. flask and made exactly to volume. Three portions, each of 10 cc., are used for the estimation of *dry matter* and the remaining 70 cc. after precipitation with basic lead acetate (usually 70–100 cc. are required) are filtered off on a Buchner funnel, and washed until the volume of the filtrate is nearly 500 cc. The excess of lead is removed by adding exactly the necessary quantity of solid sodium carbonate and the solution diluted to 500 cc. 25 cc. portions of the clear filtered solution are used to measure the *direct reducing* and

chlorophyll or fatty matter is present it is necessary to wash out the flask with a little hot alcohol or toluene; in this way there is no difficulty in transferring to the measuring flask every trace of soluble matter, whether sugars or leaf fats. The solution is then diluted to 500 cc. at 15° and is usually fairly homogeneous; when, however, toluene has been added the mixture should be thoroughly shaken so as to form a fine emulsion immediately before withdrawing each of the samples for the dry matter estimations. Working in this way the results are usually quite concordant, the differences seldom exceeding 0·2 per cent. on the weight of dry matter.

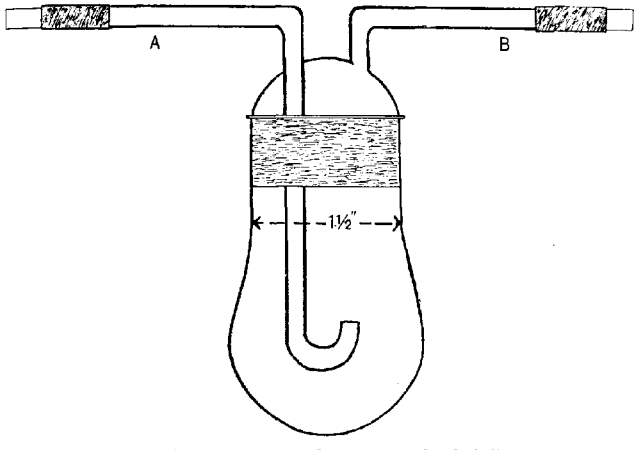

Fig. 2. Flask used to estimate dry matter in the alcoholic extract.

For the *dry matter estimations*, 20 cc. of the 500 cc. are transferred by means of a pipette to the small flask shown in Fig. 2, evaporated to dryness on a water-bath and finally dried *in vacuo* at 110°; three samples of 20 cc. should be withdrawn, two being used for the actual estimations and the third kept as a reserve in case the duplicates do not agree sufficiently closely. Usually the duplicates agree, when say 1·5 to

rotatory powers and portions of 50 cc. for inversion with citric acid and with invertase. After inversion these portions are neutralised, diluted to 100 cc. at 15° and 50 cc. of the solutions so obtained are used to measure the increase of reducing power or change of rotatory power caused by inversion. This 50 cc. corresponds with the 25 cc. used for the *direct* measurements. 50 cc. of the 500 cc. are used to estimate the *pentoses*, and 50 cc. portions are fermented with the special maltase-free yeasts to estimate *maltose*, controls being also made with baker's yeast.

2·0 grms. of dry matter are weighed, to within a few milligrams, so that the error involved in this method is considerably less than the probable error of sampling. The apparatus shown in Fig. 2 consists of a small *round*-bottomed flask with a light, ground-in glass stopper which carries two side tubes as shown, one of which, *A*, passes to within an inch of the bottom, whilst the other, *B*, only just enters the stopper. The two tubes can be closed with glass plugs as shown. The flask and stoppers are first weighed alone and then the 20 cc. of extract is evaporated in the flask as far as this is possible in the water-bath; the stopper is then inserted and the flask placed in a copper Meyer bath, filled with toluene, the end of the tube *A* being connected with the vacuum pump, a flask of phosphorus pentoxide being interposed to absorb moisture. An ordinary Meyer bath serves to heat two of these flasks at the same time, the copper lid of the bath being replaced by an asbestos cover with two holes cut in it to take the necks of the flasks. In all cases the dry matter was heated 18 hours *in vacuo* at 110°, the weight being then practically constant. Unless the drying be carried out *in vacuo* in the manner described it is impossible to obtain anything like constant results.

The apparatus shown in Fig. 2 serves also to estimate the *moisture* in the leaf matter left after extracting the sugars (see p. 268) prior to the estimation of *starch*.

Estimation of sugars. The 440 cc. remaining of the original 500 cc. are transferred to a large flask, diluted with about 300 cc. of water and *exactly* the necessary quantity of basic lead acetate[1] added to precipitate the whole of the tannins, amino-acids, etc. present; care should be taken to avoid any considerable excess of the basic lead, which must be added in small quantities at a time, until on filtering a small portion of the solution and testing it no further precipitate is produced. Working in this way it is possible to avoid having more than 1 or 2 cc. of the lead acetate solution in excess at the end of the precipitation. The quantity of lead acetate solution used[2] with different samples varies widely, according to the nature of the leaf, the time of year, etc.; when 1 kilogram of mangold leaf is used, the 440 cc. of the sugar solution requires from 200–300 cc. After the precipitation is complete the solution is filtered on a large Buchner funnel (6 ins.) and the precipitate

[1] We show in a separate paper that, by using basic lead acetate in the manner we prescribe, no loss or destruction of the sugars is to be feared.

[2] We use the ordinary solution of basic lead acetate as employed in general sugar analysis (sp. gr. 1·25, see Allen's *Commercial Organic Analysis*, vol. I. p. 308).

pressed down and washed until the filtrate and washings have a volume of nearly 2 litres. A little solid sodium carbonate is then added[1] until the lead is *exactly* precipitated, testing small portions so as to avoid any considerable excess of sodium carbonate; the solution is then diluted to 2000 cc. and a little toluene added (1 cc.) to obviate bacterial or fermentative change. We have found that a solution prepared in this way can be left for several weeks without showing any change in the proportion of sugars present; but care must be taken that the solution is not left for any length of time, even a few hours, with *any excess of basic lead acetate* or any *considerable quantity of alkali, as both of these substances rapidly destroy laevulose.*

The actual estimation of the sugars is described in a previous paper [1913, p. 466].

Polarimetric Arrangements.

With plant extracts such as those we have been studying the purified solution finally used for analysis is very dilute and therefore gives very small angular readings in the polarimeter—generally less than 1° in a 200 mm. tube. For the results to be correct within 1 per cent., the angular readings must therefore be accurate to within 0·01°. As slight differences in temperature cause considerable alterations in the specific rotatory power of laevulose and invert sugar, precautions must be taken to ensure constancy of temperature during the observations. All our readings have been taken exactly at 20·00°. By means of the following simple thermostatic arrangement it is easy to maintain this temperature to within $\frac{1}{100}$° for weeks together (Fig. 3).

The temperature of the bath *W*, which consists of a large enamelled iron vessel 16 ins. × 10 ins., is controlled by a Lowry toluene thermo-regulator *A*; the stirrer *B* is constructed from an old bicycle hub by lengthening the spindle in both directions and attaching above a wooden pulley 7 ins. in diameter, and below a four-bladed paddle. This stirrer and the small Köhler centrifugal pump *C* are run from the same small electric motor ($\frac{1}{80}$ h.p.) by a three-grooved pulley. The water-bath *W* is kept covered by a tin-plate cover, with holes and grooves cut in it to admit the neck of the thermo-regulator and other fixtures; a thermometer (not shown in drawing) graduated in hundredths of a degree also passes through this cover and enables the temperature to be read accurately to $\frac{1}{200}$° C. The water of the bath, which is maintained at a

[1] When the content of sugars is too high to allow of the analysis being made directly by Brown, Morris and Millar's method, 300 cc. of the 2 litres should be diluted to 500 cc.

constant temperature by the thermo-regulator, is circulated through the jacket of the polarimeter tube and back to the bath by means of the small centrifugal pump C; it takes only about 5 minutes to bring the temperature of the tube exactly to 20·0°.

The polarimeter we have used is one of Schmidt and Haensch's Lippich instruments, with a triple field, capable of taking a 400 mm. tube and reading in angular degrees to 0·01°; it is fitted with a spectroscopic attachment and a scale enabling the instrument to be used with mono-chromatic light of any desired wave-length. All the observations

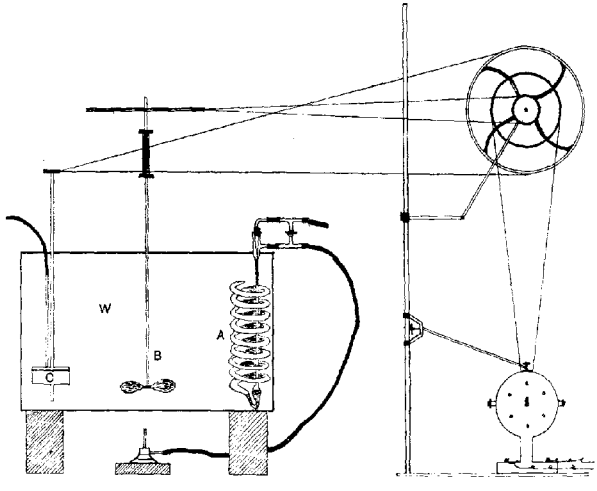

Fig. 3. Thermostat for polarimetric measurements.

recorded in the present paper were made with a sodium lamp, but for this class of work a mercury vapour lamp is preferable as it obviates the need of constantly replenishing the flame, gives a far brighter light and is much cleaner in use. We have used very successfully a Lummer-Straubel *glass* lamp, fitted with a water cooling jacket, which is preferable to the quartz mercury vapour lamps as it gives no trouble with ultra-violet rays, is far cheaper and throws a small concentrated light directly into the polarimeter tube. This lamp needs 25 volts, but can be run from an ordinary 100 volt circuit by interposing a suitable resistance: for this purpose we have used a wall type adjustable resistance with a radial switch controlling twelve contacts giving a range of 2–10 amps.

at 90 volts drop, the last three contacts giving 10 amps. at 80, 70 and 60 volts drop. This was made for us by Messrs Tyler and Freeman, London.

Experience has shown that by taking eight to ten readings it is possible with our instrument to obtain values which have a probable error considerably less than $\pm$ 0·005°. Duplicate sets of readings with a clear solution generally agree to within 0·002°.

Probable error of the Analyses and Methods of Sampling.

A. *Methods of analysis.* To ascertain whether any inversion of cane sugar is caused during the evaporation *in vacuo* and the subsequent treatment of the alcoholic extract, and also the degree of accuracy with which cane sugar can be estimated in such solutions, the original extract from a picking of mangold leaves having a volume of about 3 litres was made up exactly to 4 litres. It was then divided into two halves, each exactly of 2 litres, and to one of the halves 5·00 grms. of pure saccharose was added, to the other nothing. Each half was then evaporated *in vacuo* as usual and subjected to the ordinary processes of analysis. As a result it was found that the difference between the average values in the two cases, *by the reduction process*, using both the invertase and citric acid methods of inversion, corresponded with 4·98 grms. instead of the 5·00 grms. actually added. The *increase* of polarisation in the direct solution due to the added sugar gave a slightly higher value, 5·22 grms. saccharose, whilst the results calculated from the *change of polarisation on inversion* gave for the added sugar:

1. Invertase 5·80
2. Citric acid 6·16

} average = 5·98 grms.

The results obtained by the *change of polarisation* on inversion are therefore *nearly 20 per cent. high.* Throughout our work we have observed a similar divergence between the results obtained by the reduction and polarisation methods; the explanation of this is given later (see p. 329). Consequently in discussing the variation of the sugars throughout the day we have used the reduction values only and ignored the polarisation results, which are undoubtedly high in the majority of cases and especially so in the case of the stalks and mid-ribs. The value 4·98 obtained for saccharose by the reduction method as compared with the 5·00 grms. actually added shows that no inversion or loss of cane sugar is to be feared under our conditions of working and that the reduction method is quite trustworthy.

B. *Error of sampling.* The leaves of the mangold on different plants at any particular date are very variable in size so that some doubt might be felt as to the range of variation of the sugars due to differences of sampling. Our practice has been to pick about 120 medium-sized leaves from the plot dealt with, ignoring the very large and very small leaves. In all cases care was taken to choose leaves of a good colour and normal growth, well exposed to light; one leaf only has been taken from each root at each picking. To obtain an idea of the probable extreme error of sampling under these conditions, two lots of leaves were picked at precisely the same time (2.45 p.m., October 8th, 1914) from the same plot, at a late stage of growth when the difference between individual plants is at its greatest. These, on analysis, gave the following results:

	From reduction values		Cane sugar % from change of polarisation on inversion	
	Hexoses calculated as invert sugar % on T.V.D.M	Saccharose % by inversion on T.V.D.M.	Direct solution read when faintly alkaline	Direct solution read in presence of SO_2
First sample ...	19·0	7·52	—	8·62
Second sample	17·9	7·57	8·09	8·35

Whilst there is a very close agreement (7·52 and 7·57 per cent.) for the two samples in the case of cane sugar estimated by the change of reducing power on inversion, there is a considerably greater difference between the values for reducing sugars, calculated as invert sugar; the difference is about a single unit or about 6 per cent. of the total reducing sugars. The values obtained for saccharose from the change of rotation on inversion are, as is usually the case, from 7 to 15 per cent. higher than the reduction values; no closer agreement is obtained by taking the initial direct reading after saturating the solution with sulphur dioxide so as to have it acid and not faintly alkaline, in fact the difference is slightly *greater*. The cause of this difference is dealt with in the next paper.

The above case probably gives an extreme value for the error of sampling, as owing to the very dry autumn the leaves of most of the plants were beginning to turn yellow and were far more variable than is usual at this time of year, so that it was difficult to get a fair and representative sample. The mangolds were lifted about three weeks earlier than usual and this picking was made four days before the lifting

of the roots began. In ordinary cases, the error of sampling in the case of the saccharose estimations is probably negligible, and in the case of the reducing sugars not greater than 2 or 3 per cent. of the actual values.

RESULTS OF MANGOLD EXPERIMENTS.

The mangolds used were Sutton's Yellow Globe and were grown on Plot 9 of Barn Field[1], which is manured with minerals and nitrate of soda. The pickings were taken so as to obtain information as to the sugars in the leaves and stalks at different stages of growth. During the early period of growth the plant is mainly occupied in forming leaf, and the root is relatively small, consisting merely of a tap root and root hairs; later on the leaf formation reaches a maximum and the root then develops rapidly and stores sugar abundantly, little increase taking place in the leaves. Records of the ratio of leaf to root during growth in recent seasons are not available for the mangold, but the systematic experiments on the closely allied sugar beet carried out in France and Germany in 1913 and recorded by Vivien [1913] show that from June 17th to August 26th, whilst the leaves steadily increased in weight, the ratio of the weight of leaves to root fell from 6·06 to 1·61; during the next month, August 26th to September 30th, when growth was finally complete, the weight of the leaves was actually falling, but the root increased by large amounts. The ratio of leaves to root fell in this period from 1·61 to 1·04. When the roots are lifted the weight of the leaves is nearly the same as the weight of the roots. In the case of the mangold, which stores a much smaller proportion of sugar, the weight of leaves at lifting is always much smaller than the weight of the root—generally only about one-third to one-quarter, as shown by the data given in the note below and the records of the Rothamsted experiments. The relationship of the leaf to root in the sugar beet and mangold is in accord with the fact that in the beet the percentage of sugar is roughly three to four times that of the mangold (yellow globe), so that the ratio of leaf to root and the percentage of sugar are roughly proportional values.

Our samples were taken at three different dates, so as to give data representing three distinct stages of growth:

Series I. Early growth, when leaf formation predominates. Samples were taken every two hours during a complete period of 24 hours,

[1] This plot receives as manure 500 lbs. of potassium sulphate, 200 lbs. of magnesium sulphate, 200 lbs. of sodium chloride and 550 lbs. of sodium nitrate to the acre. The yield was on this plot in 1912: roots 17·95 tons, leaves 6·08 tons per acre; in 1913, roots 21·2, leaves 7·06 tons per acre.

starting at 6 a.m. on August 26th and ending at 4 a.m. next morning (August 27th, 1913). The seeds were sown for this crop on June 9th.

Series II. Intermediate growth, September 10th–11th, 1912, when leaf formation is relatively small and the sugars are being vigorously stored in the root, which is growing rapidly.

Series III. Final stage of growth, October 11th–12th, 1912; growth of root practically complete, roots lifted at end of October.

Method of expressing the results.

As the amount of water in the leaves and stems varies widely with the meteorological conditions, the method of analysis described above was decided upon, so that the results could be calculated upon the *total vacuum dried matter* of the material dealt with. From the data obtained it is possible also to calculate the relationship between the material soluble in alcohol and that left undissolved and also the ratio existing between the sugars at the different times of picking.

A. The Sugars of the Mangold Leaf.

Series I. Early Growth, August 26th–27th, 1913.

The results obtained in the first series of pickings (August 26th–27th, 1913) are given in Table I, and are shown graphically in Fig. 4.

Maltose and *Starch* are entirely absent from the mangold leaf throughout the day and night. This is true also of the later stages of growth[1] (see Tables II and III).

[1] We have found that although very young seedlings of the mangold store starch abundantly in the leaf, the starch disappears entirely as soon as the root begins to grow and becomes capable of storing the sugars elaborated in the leaf. It would therefore appear that the mangold has the power of forming starch but never exercises it in the later stages of growth when the sugars formed in the leaf can readily be translocated away. The leaves of very young plants appear, when examined by the chloral-hydrate-iodine method, to be gorged with starch after a bright day, probably owing to the fact that the formation of starch at this stage is of service in preventing too high a concentration of the sugars in the leaf cells which cannot be dealt with by other methods, but after about the end of July, as our analyses and microscopic tests have shown, starch is invariably absent because the sugar can then be translocated to the root and prevented from accumulating. These facts throw a clear light on the function of the formation of starch in the leaf, which clearly serves to reduce the concentration of the sugars and thus prevent it from attaining too high a value, such as would be prejudicial to the plant. In this connection the fact established in 1885 by A. Meyer is of importance, viz. that plants which store starch abundantly contain comparatively little of the reducing or non-reducing sugars, whilst leaves of plants like *Iris germanica*, *Allium cepa* and snowdrop, which form very

Saccharose and Reducing Sugars. The data given in Table I show that, on the whole, there is a close agreement between the values obtained for cane sugar by the two methods used—inversion by citric acid and inversion by invertase. The values given are those obtained by the reduction method; the results obtained by the double polarisation method, as pointed out on p. 274, are uniformly higher. The cause of this difference and of the fact that the reduction values obtained by citric acid are nearly always slightly, but only slightly, higher than the corresponding invertase figures, will be discussed in a separate paper.

Fig. 4 shows that the cane sugar and hexoses both begin to increase in amount immediately after sunrise; the increase follows more or less closely the temperature curve[1]. But whilst for the two sets of sugars the increase from 6 a.m. to 10 a.m. takes place practically along straight lines, this is not the case with the temperature, and the maximum of reducing sugar is reached at 10 a.m., considerably before the maximum either of temperature or cane sugar; the maxima of these last two curves however synchronise at about 2 p.m. During the period of daylight the cane sugar curve is roughly parallel to the temperature curve, the saccharose rising as the temperature rises and falling as the temperature falls. On the other hand, the hexoses, which at first increase more rapidly than the saccharose, subsequently, between 10 a.m. and 4 p.m., fall more abruptly than this sugar and during the night period follow almost a straight line, which is very nearly parallel to the straight line which shows the fall of the cane sugar.

The important thing to be noted with regard to these curves is their comparative simplicity as compared with the corresponding curves later in the season (see Figs. 5 and 6). No night maximum is observed

little starch in the leaf, show high concentrations of sugars (compare Parkin [1912]). As we show later, the potato, which forms starch abundantly, only contains a small proportion of sugars in the leaf.

The dependence of the starch content of the mangold leaf on the degree of development is well shown by the results obtained on examining leaves of the mangold which were plucked simultaneously on a bright day, at 11 a.m., July 15th, 1915, from plants growing on differently manured plots. Leaves from plots 5 O, 6 O, 8 O, which lack nitrogenous manure, were very small, and contained an abundance of starch; in all these cases the root was still very small. On plot 2 N (dung, super, potash and sodium nitrate) the leaves were much larger (3 ins. × 2 ins.), but still showed some starch; but on 2 A, where the leaves were much farther advanced (5 ins. × 2½ ins.) starch was practically absent. Even the guard-cells of the stomata were nearly empty.

[1] We do not wish to infer that the increase is merely a temperature effect: the rise of the sugars is probably directly related to the intensity of solar radiation and is a photochemical effect of which the temperature gives merely a rough index.

such as occurs later on, although the percentage of cane sugar actually found at 12 midnight is distinctly above the straight line drawn to indicate the falling off of the saccharose; a fact which suggests that a slight increase occurs in this sugar, similar in its nature but on a

TABLE I.

August 26th. Sun rises 5.4 a.m. Sun sets 7.0 p.m. August 27th. Sun rises 5.5 a.m. Sun sets 6.59 p.m.

Time	Temp.*	% of leaf: Soluble† in alcohol	% of leaf: Insoluble in alcohol	Sacc. % in T.V.D.M.: Citric acid	Sacc. % in T.V.D.M.: Invertase	Δ = C.A. − I.	Saccharose av. %	Hexoses % as I.S.‡	Saccharose + hexoses	Pentose %	Pentosan %	Maltose	Starch	I.S./C.S. (av.)	Remarks
6 a.m.	45° F.	42·5	57·5	2·56	2·47	+0·09	2·51	0·77	3·28	0·37	5·38	0·00	0·00	0·307	Mists clearing away
8 a.m.	62°	42·5	57·5	2·76	2·75	+0·01	2·75	1·42	4·17	0·42	5·33	,,	,,	0·517	Brilliant sunshi
10 a.m.	70°	40·8	59·2	3·02	3·05	−0·03	3·04	2·16	5·20	0·44	5·65	,,	,,	0·710	,, ,,
12 noon	73°	40·6	59·4	3·17	3·04	+0·13	3·11	2·15	5·26	0·41	5·53	,,	,,	0·691	,, ,,
2 p.m.	75° (max.)	37·3	62·7	3·23	2·96	+0·27	3·09	1·94	5·03	0·41	5·61	,,	,,	0·628	Bright sun
4 p.m.	71°	37·2	62·8	3·09	3·01	+0·08	3·05	1·18	4·23	0·41	5·39	,,	,,	0·387	Still quite brig but slightly ha
6 p.m.	66°	40·1	59·9	2·96	2·68	+0·28	2·82	0·96	3·78	0·45	5·19	,,	,,	0·341	Bright sunshin
8 p.m.	61°	37·1	62·9	2·47	2·22	+0·25	2·35	0·90	3·25	0·36	5·50	,,	,,	0·383	Just dark
10 p.m.	58·5°	40·4	59·6	2·22	2·09	+0·13	2·15	0·74	2·89	0·37	5·31	,,	,,	0·344	Dry
12 mid-night	58°	38·0	62·0	2·41	1·96	+0·45	2·18	0·57	2·75	0·42	5·65	,,	,,	0·261	Dry, but cloud
2 a.m.	57°	38·6	61·4	1·70	1·46	+0·24	1·58	0·38	1·96	0·40	5·64	,,	,,	0·241	Cloudy
4 a.m.	56° (Min. 54° at 5 a.m.)	38·0	62·0	1·71	1·28	+0·43	1·50	0·20	1·70	0·52	5·96	,,	,,	0·133	Slight rainfall 4 a.m.
6 a.m.	54·5°	—	—	—	—	—	—	—	—	—	—	—	—	—	First dawn = 4 a.m.
8 a.m.	57°	—	—	—	—	—	—	—	—	—	—	—	—	—	

* The temperatures given are the temperatures of the air recorded by the shaded automatic-recording instrument near Barn Field, 5 ft. above the ground.

† These values give the percentage of vacuum-dried solids of the leaf which are soluble in alcohol, the percentage being calculated on the *total* vacuum-dried matter of the leaf.

‡ The reducing sugars are here calculated as invert sugar after allowing for the pentoses present. If the separate amounts of dextrose and laevulose are calculated (see p. 318) the *sum* of the results, as a rule, differs only very slightly from the values given here. The question of the dextrose-laevulose ratio is discussed in a separate paper (see p. 327).

smaller scale than the increase which is found at this time of night in the later stages of growth. No stress can, however, be laid upon

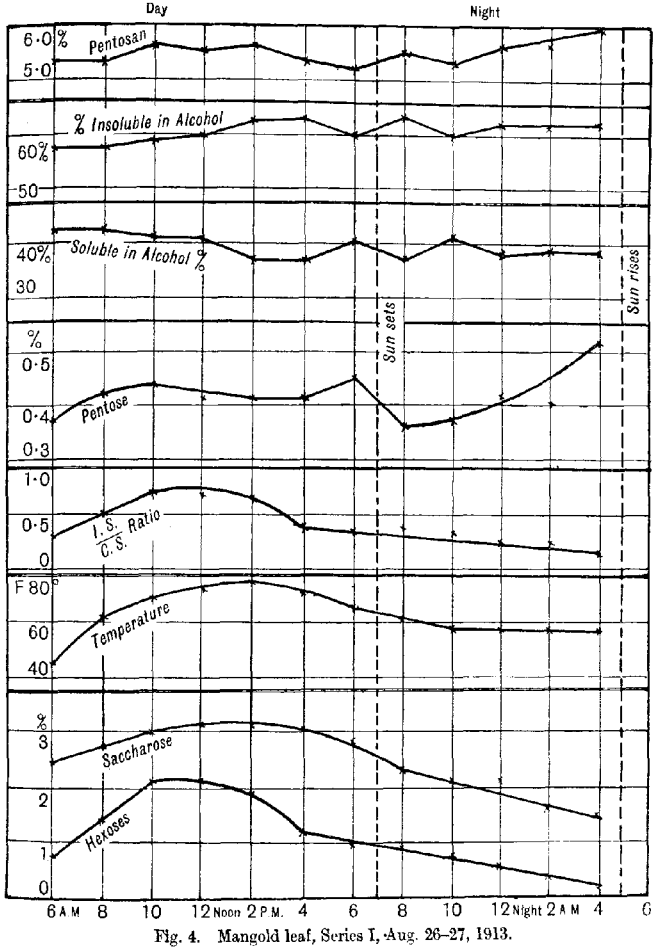

Fig. 4. Mangold leaf, Series I, Aug. 26–27, 1913.

this slight departure from a regular falling off in the sugar, as it might well be occasioned by error of analysis or sampling. That this error is, however, in general quite small may be inferred from the regular

course pursued by the curves in question. The total variation of the cane sugar during the day is only from 2·5 to 3·1 per cent., but the methods of working adopted are sufficiently delicate to show a regular and progressive variation between these extremes.

The outstanding features of the sugar curves are:

1. The rise of sugar which first occurs, followed by a rapid falling off along approximately straight lines. Practically the whole of the reducing sugar disappears during the night, but the saccharose only falls to about one-half of its maximum value (3·1 per cent. falls to 1·5 per cent.).

2. The quantity of saccharose is always greater than that of hexose sugar (seven times as great at 4 a.m., 1·5 times as great at 10 a.m.), so that the saccharose curve is always well above the hexose curve. But the hexoses increase at first more rapidly than the saccharose and later on fall off more quickly. The curve showing the ratio of hexoses to saccharose $\left(\frac{\text{I.S.}}{\text{C.S.}} \text{ ratio}\right)$ is itself more or less closely parallel to the temperature curve, a fact which becomes even more strikingly marked in Series II. Thus the proportion between the sugars—the increase of the hexoses relatively to the saccharose—seems itself to be a function of the temperature, or perhaps of the photo-chemical activity of which the temperature is in this case a rough measure. We shall discuss this point later (see p. 312).

3. The total fluctuations of the sugars are very small, especially in comparison with those found later in the season.

The saccharose increases from 2·5 per cent. to 3·11 per cent. and falls at night to 1·50 per cent.

The hexoses increase from 0·77 per cent. to 2·16 per cent. and then fall at night to 0·20 per cent.

The fluctuation of the hexoses is far greater than of the saccharose.

Pentosan Curve and Curve of Matter Insoluble in Alcohol.

From Fig. 4 it is seen that in spite of the considerable *increase* in the sugars which occurs during the day from 6 a.m. to 2 p.m., there is simultaneously a marked increase in the percentage of matter *insoluble* in alcohol; this increase runs closely parallel with an increase in the *pentosans*. The curve of insoluble matter in fact closely resembles the pentosan curve, a resemblance which becomes far more strongly marked in the September picking (see Fig. 5, p. 283), when the curves are

practically identical in form. In Fig. 4 the rise and fall of matter insoluble in alcohol which occurs just before and after dark (6 p.m. to 10 p.m.) is accompanied by a corresponding rise and fall of pentosan; the increase of pentosan at this point seems to be associated with the sudden falling off of free pentoses, which occurs between 6 and 8 p.m., but the pentoses subsequently rise during the night, side by side with the rise of pentosans and of matter insoluble in alcohol.

The increase in the amount of pentosan and of matter insoluble in alcohol which is visible during the day in spite of the increase in the amount of substances soluble in alcohol is partly due to the formation of new ligneous tissue, but is probably more the result of the formation of gummy substances, which we have found always to be present in considerable quantity in the leaf tissue. These gums, as we shall show later, probably play the part of reserve substances (see p. 285).

Although the pentosan does not vary within very wide limits (5·2 to 5·96 per cent.), the value at the end of the 24 hours (5·96) is considerably higher than at the commencement (5·38); a similar but even larger increase is found at the September picking (see Table II and Fig. 5). This is probably due to the increase of the ligneous constituents of the leaf. In October when the pentosan has increased to about 7 per cent., and the leaves are no longer growing, there is very little variation during the day (6·89 to 7·15) other than can be accounted for by the much wider variations of the total sugars.

Pentoses. Between 6 a.m. and 4 p.m. there is a slight rise in the pentoses, followed by a slight fall, the curve running more or less parallel with the other sugar curves. Between 4 p.m. and 8 p.m. there is an abrupt rise of the pentose followed by an abrupt fall, these changes synchronising with a fall and a rise respectively of pentosan. During the night the pentose rises fairly steadily, and the same is true of the pentosan, both changes apparently taking place at the expense of the saccharose and reducing sugars, which are falling steadily throughout the night—especially the hexoses, which practically disappear. These facts and the parallelism of the pentose curves with those of the other sugars during the day, suggest that the pentoses arise from the reducing sugars and the pentosans from the pentoses.

At this stage of growth, when leaf formation is predominant, it is interesting to note that the pentoses (0·41 per cent.) at their midday maximum have roughly the same ratio to the pentosan tissue (5·5 per cent.) as the other sugars (5·2 per cent.) have to the total insoluble leaf material (60 per cent.), the ratio being roughly $\frac{1}{12}$.

The total fluctuation during the 24 hours is small—ranging only from 0·37 to 0·52 per cent.

Series II. Intermediate Stage of Growth.

TABLE II.

Mangold Leaves, September 10th–11th, 1912.

Sept. 10th. Sun rises 5.28 a.m. Sept. 11th. Sun rises 5.30 a.m.
Sun sets 6.26 p.m. Sun sets 6.24 p.m.

Time	Temp.	% of leaf*		Sacc. % in T.V.D.M.		Δ = C.A. − I.	Saccharose av. %	Hexoses % as I.S.†	Saccharose + hexoses	Pentose %	Pentosan %	Maltose	Starch	I.S. / C.S. (av.)	Remarks
		Soluble in alcohol	Insoluble in alcohol	Citric acid	Invertase										
10 a.m.	50° F.	54·7	45·3	4·60	4·43	+0·17	4·51	5·72	10·23	0·34	4·42	0·00	0·00	1·27	Dull, cold wind
1 p.m.	53°	44·2	55·8	4·86	4·57	+0·29	4·62	7·50	12·12	0·39	5·74	,,	,,	1·59	Dull, cold wind
4 p.m.	50°	48·5	51·5	4·41	4·35	+0·06	4·38	7·00	11·38	0·68	5·25	,,	,,	1·60	Dull, slight rain
6 p.m. Dark 7 p.m.	49°	51·0	49·0	6·46	6·32	+0·14	6·39	8·90	15·29	0·45	5·18	,,	,,	3.30 1·39	Dull, slight rain
8 p.m.	47°	47·2	52·8	5·61	5·27	+0·34	5·44	6·76	12·20	0·71	5·52	,,	,,	1·24	Clear, starlight
11 p.m.	43°	49·8	50·2	6·35	6·47	−0·12	6·41	7·10	13·51	0·71	5·31	,,	,,	1·11	,, ,,
2 a.m.	44°	50·7	49·3	8·28	8·26	+0·02	8·27	7·81	16·08	0·76	5·29	,,	,,	0·94	Cloudy
4 a.m. Light 5 a.m.	44°	51·3	48·7	5·68	5·57	+0·11	5·62	6·91	12·53	0·62	5·26	,,	,,	1·23	Cloudy
6 a.m.	44°	47·5	52·5	4·23	4·24	−0·01	4·24	6·30	10·54	0·65	5·67	,,	,,	1·48	Overcast
8 a.m.	46°	45·7	54·3	4·79	4·42	+0·37	4·60	5·38	9·98	0·61	5·90	,,	,,	1·17	,,

* Calculated on total vacuum-dried matter of the leaf.
† After allowing for the pentoses present.

Changes during the 24 hours.

The results are shown graphically in Fig. 5.

Maltose and Starch. These are absent throughout the whole 24 hours.

Saccharose and Hexoses. As in Series I the results of the inversion with citric acid are slightly higher than those obtained with invertase. The curves, both of saccharose and reducing sugars, are far more complicated than those of the first picking (August 26th). Both curves

show three well-defined synchronising maxima and minima; the maxima are at 2 p.m., 6 p.m. and 2 a.m. During the greater part of the day, viz. from sunrise to 4 p.m., the saccharose fluctuates in almost exactly

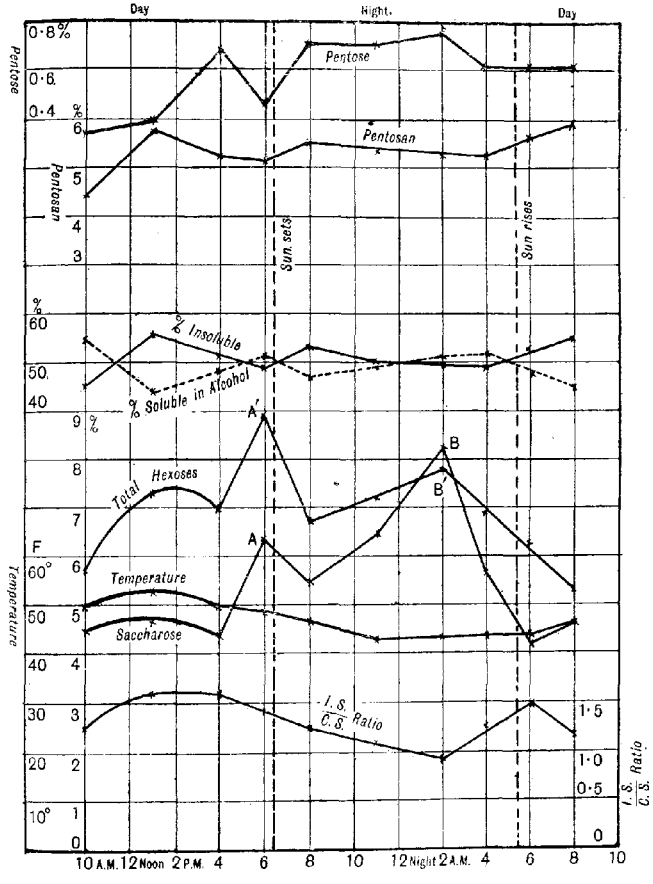

Fig. 5. Mangold leaf, Series II, Sept. 10–11, 1912.

the same way as the temperature. The actual change in the amount of sugar is relatively small, probably owing to the fact that on this particular day there was a lack of direct sunshine, and a cold wind

prevailed; the rise of temperature (50–53°) was slight from 10 a.m. to 1 p.m., when the maximum was reached, and the corresponding increase in saccharose was very small, viz. from 4·51 to 4·62 per cent. On the other hand the increase of hexoses takes place far more rapidly, as was the case in August, but with this exception, the general *shape* of the hexose curve is the same as that of the saccharose curve. In both cases, two peculiar maxima appear (at 6 p.m. and 2 a.m.) which were not found at the earlier stage of growth, but reappear, as we shall see later (see Fig. 6), in October. Both the cane sugar and reducing sugar after reaching a maximum at about 2 p.m., corresponding with the temperature maximum, begin to fall off slightly up to 4 p.m., when a sudden rise in both sugars occurs, just *before* sunset, maxima A and A' being reached which are considerably higher (about 50 per cent.) than the highest values previously reached. Similar maxima are found in the October picking (Fig. 6) just *after* sunset. These sudden increases in the sugars at this time of day find a parallel in the potato leaf (see p. 366, and Fig. 1) in an equally sudden increase in the starch (from 2 to 6 per cent.). It may be that the rise in the sugars may be accounted for by a cessation in their translocation from the leaf, leading to an accumulation in the leaf tissue. But from 6 p.m. to 8 p.m. both sugars are again falling; at 8 p.m. a second rise sets in, which is more rapid in the case of the saccharose than of the hexoses, the maximum B for cane sugar being actually higher than that for the reducing sugars, B'. At this point the percentages of saccharose and hexoses are nearly the same, viz., about 8 per cent.; the percentage of cane sugar at this point is far higher than at any previous hour of the day, being nearly double that corresponding with the maximum reached at 2 p.m. when the leaves were exposed to direct light, and $1\frac{1}{3}$ times the value reached at 6 p.m. Just as the night rise of saccharose was more rapid than that of the hexoses, the falling off of the cane sugar is also more abrupt. It is noteworthy that the increase of hexoses from 8 p.m. to 2 a.m. and the subsequent decrease from 2 a.m. to 8 a.m. take place along exactly straight lines; with the cane sugar this is not strictly the case. The fall of hexose continues also some little time after sunrise, but the saccharose apparently responds at once to the daylight and increases in amount.

It is a very striking fact that although the curves for saccharose and the hexoses are of so complicated a character, the curve showing the variation of the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ (that is invert sugar to cane sugar) is relatively

simple. As in the earlier stage of growth (see Fig. 4) the curve more or less closely follows the temperature curve during the day and the greater part of the night. As the temperature rises from 10 a.m. to 1 p.m., the invert sugar increases faster than the saccharose, but when the temperature falls, the ratio of invert sugar to cane sugar falls off along *practically a straight line*, exactly as was the case in Series I (Fig. 4). From 2 a.m. to 6 a.m., the saccharose is disappearing faster than the hexoses, so that the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ rises, again along nearly a straight line, until just after sunrise, when the formation of cane sugar begins more rapidly than that of hexose sugar.

It is very difficult to explain the night maxima, B and B', which form such a striking feature of the sugar curves at this stage of growth and also in the later and final period in October (see Fig. 6, p. 289). The maxima reached at night are in the case of both sugars considerably higher than those attained during actual insolation. Both sugars increase together and fall together, so that interconversion cannot explain the result. The *sum* of the sugars at the night maximum (16·6 per cent.) is slightly higher than at 6 p.m. (15·3 per cent.) and far higher than at 1 p.m. (12·1 per cent.), when the direct formation of the sugars under the influence of light reaches a maximum. It is improbable that, at night, a reverse current of sugar sets in from the roots to the leaves and our actual analyses as well as a careful microscopic examination of all the samples have shown the entire absence of starch from the leaf during the day and night. Had starch been present, the increase in the amount of the sugars at night might be due to the transformation of starch into these. In the absence of starch, any explanation of the large increase in the proportion of soluble sugars which is observed to attain a maximum in the neighbourhood of 2 a.m. (at about 3 a.m. in October, in both cases about 3 hours before sunrise) must be more or less conjectural. Both in September (Series II) and October (Series III) the maximum concentration of the sugars is reached at nearly the same time, whilst the proportion of cane sugar is practically identical (about 8 per cent.) in both cases, in spite of the day values for saccharose being far lower in Series II than in Series III. As starch and the product of its hydrolysis, maltose, are entirely absent from the mangold leaf it seems probable that some other substance acts as a reserve at this period of growth, and, during the night, is broken down to cane sugar and invert sugar, thus causing the rise which is observed. The mangold leaf undoubtedly contains a large amount of *gummy* substance which is soluble in water

and, after the attempted conversion of the starch with taka-diastase, is precipitated as a semi-crystalline mass by basic lead acetate. Whether this substance can give rise directly to cane sugar or reducing sugars on hydrolysis can only be decided by a special investigation, but it would appear that this or some kindred substance is the source of the great increase of sugars which occurs between midnight and 3 a.m., both in the September and October pickings.

As compared with the earlier picking in August the following are the outstanding features:

1. The proportions and range of variation of the sugars are considerably greater:

On August 26th–27th, the saccharose varied from 3·05 to 1·5 per cent., hexoses from 2·15 to 0·2 per cent.

On September 10th–11th the saccharose varied from 8·27 to 4·24 per cent., hexoses from 8·9 to 5·4 per cent.

This, too, in spite of the fact that September 10th was a dull, cool day unfavourable to photo-synthesis.

2. The relative position of the saccharose and hexose curves has changed; whereas in August the cane sugar curve was always *above* the hexose curve, the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ varying between the limits 0·13 and 0·71, in no case reaching 1·0, on September 10th–11th the hexose curve is throughout the 24 hours above the cane sugar curve, except for a moment at 2 a.m., when the two sugars are present in nearly equal amounts. During the 24 hours the $\frac{\text{I.S.}}{\text{C.S.}}$ varies from 0·94 to 1·60.

3. During the later period of the night, when the proportion of sugars is falling, the leaves became nothing like so depleted of sugars as in the earlier stage of growth; whereas on August 26th–27th the cane sugar fell to 1·5 per cent. and the hexoses practically disappeared, on September 10th–11th the lowest value reached by the cane sugar was 4·24 per cent. and by the hexoses 5·4 per cent.

Pentosans, Matter Insoluble in Alcohol and Pentoses.

As at the August picking, but in a far more unmistakable manner, the curve showing the proportion of leaf substance which is insoluble in alcohol runs *exactly parallel to the pentosan curve* (see Fig. 5). It is a striking fact that from 10 a.m. to 1 p.m., in spite of the large increase in the sugars, that is of matter soluble in alcohol, there is a large actual increase of substances which are insoluble in alcohol, and exactly

parallel with it, a rise in the pentosans. It is clear therefore that the increase of pentosan material, which probably includes the gum-like substances mentioned above (p. 285), must take place at a relatively greater rate than the increase of sugars, since their increase does not mask it. Saccharose, hexoses, pentoses and pentosans are all increasing simultaneously during the first period of the day, that is whilst the temperature is rising; it is probable, as stated on p. 281, that the hexoses are converted into pentoses and the latter into pentosans. Thus we find the pentoses rising not so quickly as the pentosans whilst the latter are being formed, but from 2 p.m.–4 p.m. when the hexoses are *falling* a rapid rise of pentoses occurs, whilst the pentosans from this point up to 6 p.m. have ceased to be formed. It is probable that the sudden apparent *fall* followed by a *rise* of pentoses between 4 p.m. and 8 p.m. is partly a relative effect owing to the large sudden increase and decrease of the saccharose and hexoses between these points; but this would not account for the magnitude of the change (from 0·68 to 0·45 per cent. and back again to 0·71 per cent.), and the two changes are obviously interconnected and take place in opposite directions. From 8 p.m. to 2 a.m. there is an actual rise of pentoses, which must be somewhat greater than it appears because it is partly masked by the large increase of saccharose and hexoses during this interval. The fall of pentosans between 8 p.m. and 4 a.m. is probably only an apparent or relative effect, owing to the large increase in the other sugars, but the rapid rise of pentosans from 4 a.m. onwards corresponds with the actual fall of pentoses from 2 a.m. onwards, which must be larger than it appears because the saccharose and hexoses are falling simultaneously.

The actual range of pentoses during the day is small, viz. 0·34–0·76 per cent.

Series III. Final Stage of Growth, October 11th–12th, 1912.

Changes during the 24 *hours.*

Maltose and *Starch* are again entirely absent.

Saccharose and Hexoses. These increase immediately after sunrise, the curves (see Fig. 6) rising at first almost parallel to the temperature curve; the cane sugar increases in amount until midday but from this point until sunset (5 p.m.) the *total* quantity of sugars present remains nearly constant, the fluctuations between 11 a.m. and 5 p.m. consisting merely of interconversions of the cane sugar and invert sugar. Thus between 1 p.m. and 3 p.m. cane sugar increases, apparently at the expense

of the reducing sugars, whilst from 3 to 5 p.m. cane sugar falls and the hexoses increase in amount. At sunset, as in the August picking, a rapid rise of both cane sugar and reducing sugars occurs, the cane sugar reaching its maximum first at 7 p.m. and then apparently being converted

TABLE III. *Mangold Leaves*, October 11th–12th, 1912.

Oct. 11th. Sun rises 6.19 a.m. Oct. 12th. Sun rises 6.21 a.m.
Sun sets 5.15 p.m. Sun sets 5.13 p.m.

Time	Temp.	% of leaf*		Sacc. % in T.V.D.M.		Δ = C.A. − I.	Saccharose av. %	Hexoses % † as I.S.	Saccharose + hexoses	Pentose %	Pentosan %	Maltose	Starch	I.S. / C.S. (av.)	Remarks
		Soluble in alcohol	Insoluble in alcohol	Citric acid	Invertase										
9 a.m.	40° F.	52·2	47·8	5·67	5·38	+0·29	5·53	10·32	15·85	0·82	6·89	0·00	0·00	1·87	Foggy night, a getting thro at 9 a.m.
11 a.m.	55°	54·6	45·4	7·29	7·02	+0·27	7·16	11·62	18·78	0·91	6·21	,,	,,	1·62	Warm and sunny
1 p.m.	61°	52·5	47·5	7·19	6·83	+0·36	7·01	12·12	19·13	0·92	6·59	,,	,,	1·73	Very sunny
3 p.m.	58°	54·9	45·1	8·92	9·11	−0·19	9·02	10·24	19·26	0·86	6·35	,,	,,	1·14	Sunny
5 p.m.	50°	52·5	47·5	7·64	7·41	+0·23	7·52	11·46	18·98	0·86	6·68	,,	,,	1·52	Cooler, haze
Dark 6.30															
7 p.m.	44°	54·0	46·0	9·48	9·56	−0·08	9·52	11·47	20·99	0·92	6·60	,,	,,	1·20	Hazy
9 p.m.	42°	54·2	45·8	7·41	7·16	+0·25	7·28	11·98	19·26	0·84	6·65	,,	,,	1·65	Hazy
11 p.m.	39°	47·9	52·1	6·80	6·78	+0·02	6·79	9·39	16·18	0·68	7·15	,,	,,	1·38	Slight fog
1 a.m.	38°	52·6	47·4	7·15	6·68	+0·47	6·92	10·78	17·70	0·80	7·09	,,	,,	1·56	Cold and fog
3 a.m.	36°	55·9	44·1	8·40	8·42	−0·02	8·41	12·41	20·82	0·70	6·78	,,	,,	1·48	Ice on leave
5 a.m.	31°	54·7	45·3	6·93	6·88	+0·05	6·91	11·49	18·40	0·70	6·77	,,	,,	1·67	Ice thick on leaves
1st light 5.30															
7 a.m.	35°	51·6	48·4	5·08	4·88	+0·20	4·98	9·62	14·50	0·61	6·77	,,	,,	1·93	Leaves from stiff

* Calculated on the total vacuum-dried weight of leaf.

† Allowance has been made for the pentoses present.

into hexoses; this is shown by the rise of hexoses between 7 and 9 p.m., whilst the saccharose is falling. From 9 p.m. to 11 p.m. both sugars are falling, but between 11 p.m. and 3 a.m. there is again a rapid rise in the sugars, exactly as in the September picking, until the night maxima, *B* and *B'*, are reached, at 3 a.m., that is 3 hours before sunrise, a position

which agrees very closely with that found in Series II. After these maxima have been reached (probably as in the August picking, owing to certain reserve substances, such as gums, being put under contribution), the sugars continue to fall at almost parallel rates and along practically straight lines until just after sunrise.

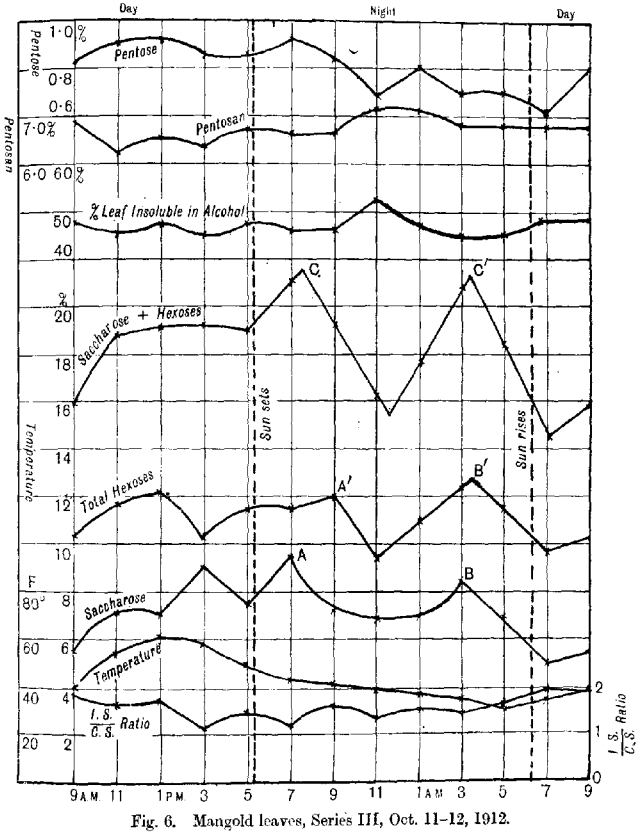

Fig. 6. Mangold leaves, Series III, Oct. 11–12, 1912.

It is a striking fact that the two maxima for the *total* sugars (*C* and *C'*) at night correspond with roughly the same proportion of sugars (about 21 per cent. of the total vacuum-dried matter); the same was true of the two night maxima of Series II, but here the value for the total

sugars was much lower, viz. about 16 per cent. It is also striking that the successive rises and falls in the proportion of *total* sugars at night occur along practically straight lines (a phenomenon also observed in Series II, although the curve of total sugars is not shown in Fig. 5).

Fluctuation of the $\frac{I.S.}{C.S.}$ *ratio.*

In the final stage of growth in October, although the general type of the sugar curves is quite similar to that of Series II, the curve showing the ratio $\frac{I.S.}{C.S.}$ is of an entirely different character. Whereas in Series I and II, during the greater part of the day, the curve showing the ratio of the two sugars runs very nearly parallel to the temperature curve, in the last stage of growth the curve shows a periodic increase and decrease of the ratio which the two sugars bear to one another. From 9 a.m. to 11 a.m. saccharose increases faster than hexoses, from 11 a.m. to 1 p.m. the reverse is true; then comes a rapid increase of saccharose and a fall of hexoses until 3 p.m., which is reversed between 3 and 5 p.m. At night there is a similar periodic fluctuation, and, exactly as in Series II, when the cane sugar increases at night it does so far more rapidly than the hexoses, cane sugar apparently being the primary product which is formed from the reserve substance, and not hexoses. On the other hand, exactly as in September, the saccharose falls off, from 3 a.m. to sunrise, considerably more rapidly than the invert sugar, so that the curve $\frac{I.S.}{C.S.}$ rises steadily; as in Series II, the rise is almost along a straight line. This is perhaps due to the inversion of the cane sugar being effected by invertase more rapidly than the invert sugar formed is consumed by the changes to which it is subjected. It is noteworthy that these changes, in this case, take place when the external temperature is below freezing-point; at 3 a.m. ice was forming on the leaves and at 7 a.m. they were all frozen stiff.

Pentosans, leaf-matter insoluble in Alcohol and Pentoses. As in Series I and II, the curve of matter insoluble in alcohol is parallel to the pentosan curve. At this stage of growth, however, the variation during the day is much less marked than in September (Series II), the insoluble matter ranging from 45–48 per cent., the pentosan from 6·2 to 6·9 per cent.; the greater part of the variation seems to be purely relative, that is the apparent fall of insoluble matter between 9 a.m. and 11 a.m. corresponds with the large increase of sugars (15·8 to 18·8

per cent.) between these hours. This is very different from the August and September pickings when a rapid formation of the pentosans (gums or ligneous tissue) was clearly obvious in spite of the simultaneous increase in the sugars. A certain amount of pentosan formation does apparently occur, however, even at this later stage of growth, and is visible in the rise between 11 a.m. and 1 p.m. The fluctuations at night seem to be principally relative—thus the increase in the matter insoluble in alcohol which occurs between 7 p.m. and 11 p.m. is due to the rapid fall of sugars from 21 to 16 per cent., but as there is a fall of the pentose curve between the same points, it appears that some real pentosan formation occurs from these sugars. The fall of pentosan and insoluble matter from 11 p.m. to 3 a.m. is also a relative effect due to the increase of the sugars and the rise from 3 a.m. to 7 a.m. is also relative, owing to the falling off of these.

The free *pentoses* increase during the day as in the earlier pickings and follow very largely the invert sugar curve; thus there is a continuous increase from 7 a.m. to 1 p.m., after which the pentose falls, apparently giving rise to pentosan. At night the pentose falls between 7 and 11 p.m. and the pentosan rises; the sudden rise of pentose between 11 p.m. and 1 a.m. occurs simultaneously with the sudden increase of hexoses. The fall of pentoses subsequently to this, from 1 a.m. to 3 a.m., is partly a relative effect, due to the rise in the reducing sugars.

The total variation of the pentoses during the day is only very small, viz. 0·82 to 0·92 per cent., but at night the fluctuations (probably largely relative) are from 0·92 to 0·61. During the day the fluctuations are considerably less than during the daytime in Series II; but at night the changes are greater, mainly representing a falling off in the pentoses.

Comparison of Series III with Series II.

1. The actual proportions of saccharose and hexoses and the range of variation of these sugars are considerably greater in Series III (October 11th–12th) than in Series II (September 10th–11th) and therefore far greater than in Series I (August 26th–27th).

September 10th–11th. Cane sugar varied from 8·27 to 4·24 per cent.; hexoses, 8·9 to 5·4 per cent.

October 11th–12th. Cane sugar varied from 9·52 to 4·98 per cent.; hexoses, 12·41 to 9·39 per cent.

In both series the cane sugar varies between wider limits than the hexoses, and the difference is most marked in Series III.

2. As in the September picking the curve of hexoses is always well above the curve of saccharose, the hexose being always largely in excess of the cane sugar. Consequently, the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ is always greater than unity; it fluctuates between the values 1·14 and 1·93; in September it ranged between 0·94 and 1·60, whilst in August the range was 0·13 to 0·71.

3. Series III differs from Series II mainly in the fact that during the earlier part of the day (9 to 11 a.m.) the saccharose increases faster than the hexoses, as shown by the falling $\frac{\text{I.S.}}{\text{C.S.}}$ ratio; subsequently the total sugars remain nearly constant in amount until after sunset. The periodic fluctuation of the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ and the mutual interconversion of cane and invert sugar which it expresses are characteristic of the final stage of growth only. Whereas, too, in the earlier pickings the proportion of the *total sugars* falls off rapidly after the temperature maximum has been reached until the rise occurs in the neighbourhood of sunset, in this case the total sugars remains nearly constant during the whole afternoon, a balance being reached such that the sugars removed from the leaf are equal in quantity to those being formed in it. This characteristic of the last stages of growth is apparently due to the root having relatively less capacity to remove the sugars from the leaf than at earlier stages; in August and September the root was capable of removing the sugars from the leaf in the afternoon faster than they were formed; in October, the out-take and production just balance one another.

4. The total sugars present are at a far higher level in Series III than in Series II; in Series II they formed 10 to 16 per cent., and in Series III from 15 to 21 per cent. of the total vacuum-dried leaf matter.

Variations during Complete Period of Growth.

In Table IV we give the diurnal variations of the leaf carbohydrates for the three stages of growth investigated.

The principal conclusions which can be drawn as to the total variations during growth are as follows:

1. The proportions of all the sugars present in the leaves increase progressively from the first to the final stage of growth; this is true of saccharose, hexoses and pentoses. The extreme diurnal variation is, however, greatest for the hexoses, pentoses, pentosan and matter

insoluble in alcohol, at the September picking (Series II); it would probably have been the same also for the saccharose had not the day been abnormally dull and cloudy, so that the range of temperature was exceedingly small ($\Delta = 7°$) and the increase of cane sugar (which follows the temperature curve) correspondingly small (see Fig. 5).

TABLE IV. *Range of variations during growth.*

Series and date	Temp. °F.	Saccharose %	Hexoses as invert sugar %	Pentose %	Pentosan %	Ratio $\frac{\text{I.S.}}{\text{C.S.}}$	Matter insoluble in alcohol %	Saccharose + hexoses %
I. Aug. 26–27	45–75 Δ=30	1·50–3·11 Δ=1·61	0·20–2·16 Δ=1·96	0·36–0·52 Δ=0·16	5·19–5·96 Δ=0·77	0·13–0·71 Δ=0·58	57·5–62·9 Δ=5·4	1·70–5·26 Δ=3·56
II. Sept. 10–11	43–50 Δ=7	4·24–8·27 Δ=4·03	5·38–8·90 Δ=3·52	0·34–0·76 Δ=0·42	4·42–5·90 Δ=1·48	0·94–1·60 Δ=0·66	45·3–55·8 Δ=12·5	9·98–16·08 Δ=6·1
III. Oct. 11–12	31–61 Δ=30	4·98–9·52 Δ=4·54	9·39–12·41 Δ=3·02	0·61–0·92 Δ=0·31	6·21–7·15 Δ=0·94	1·14–1·93 Δ=0·79	45·1–52·1 Δ=7·0	14·5–20·99 Δ=6·49

2. The proportion of *pentosan* appears to fall slightly from the first to the second Series, but then increases from the second to the third. The apparent fall is really a relative effect, due to the large increase in sugars and other soluble substances which are formed between the dates of the first and second pickings. If the pentosans are calculated as percentages of the vacuum-dried leaf matter which is insoluble in alcohol, we get a steady increase in the amount of pentosan constituent as the season advances, as follows:

Series I. Pentosans form 8·58–9·61 per cent. of the insoluble leaf matter.

Series II. Pentosans form 9·83–10·85 per cent. of the insoluble leaf matter.

Series III. Pentosans form 13·70–15·35 per cent. of the insoluble leaf matter.

In passing from Series II to Series III, a very large increase in the proportion of pentosan constituents occurs, pointing probably to an increase in lignification during the interval.

3. The *hexoses* more and more predominate in the leaf as the season advances; at first they form only a fraction of the saccharose $\left(\frac{\text{I.S.}}{\text{C.S.}}\right.$ varies from 0·13 to 0·71 in Series I$\left.\right)$, but later on they become equal

to and even nearly double the saccharose $\left(\frac{\text{I.S.}}{\text{C.S.}} = 0{\cdot}94\text{–}1{\cdot}60\right.$ in September, and 1·14 to 1·93 in October$\left.\right)$.

An exactly similar increase in the proportion of reducing sugars was observed by Parkin [1912] in the case of the snowdrop (*Galanthus nivalis*).

4. Whereas in the first stage of growth practically *all* the reducing sugar and about one-half of the cane sugar are used up in the night, in the later stages of growth only a small part of these sugars disappears in the night, so that each day's activity commences with a larger proportion of total sugars. This is well seen from the following data:

	% hexoses	% saccharose
At sunrise, August 27th	0·20	1·50
„ September 11th	6·30	4·24
„ October 12th	9·62	4·98

The store of reducing sugars which is thus available at the commencement of the day steadily and rapidly grows, especially in the earlier part of the season; but the store of cane sugar in the leaf, although increasing rapidly from August 27th to September 11th seems to reach a nearly stationary value. Table IV shows that the *limits* between which the sugars vary constantly rise during the season, but not so much in the case of the cane sugar as in that of the reducing sugars; but the range of variation *for the* 24 *hours* increases considerably more in the case of the saccharose than in that of the hexoses. It must be noted, however, that the range of variation during the daylight period, up to the time of reaching the first maximum (which corresponds with the temperature maximum), is always greater in the case of the hexoses than the saccharose, and especially so in the first two series; when growth of the root is nearly complete, the range of variation of the cane sugar in the leaf during the period of illumination becomes far greater. In this case, when the root has nearly reached the limit of its storing capacity, the leaf itself seems to act as a temporary reservoir of cane sugar. This is shown by the following data:

Daylight Variations.

	Saccharose	Hexoses
Series I	2·51–3·11	0·20–2·16
	Δ=**0·6** %	Δ=**1·96** %
Series II	4·24–4·62	5·38–7·50
	Δ=**0·38** %	Δ=**2·12** %
Series III	4·98–7·16	9·62–12·12
	Δ=**2·18** %	Δ=**2·50** %

B. The Sugars of Mid-ribs and Stalks (Petioles). The Translocation of the Sugars.

Series I. August 26th–27th, 1913.

In this series, the leaf-stalks (petioles) were divided into top and bottom halves, and the two sets were treated separately; in this way it was hoped that any change in the saccharose and hexoses during their passage to the root might be detected. Actually it was found that very considerable differences exist in the composition of the sap in the two halves, as shown by the data in Table V.

Table V. *Series I.* August 26th–27th, 1913.

Top and Bottom Halves of Mangold Leaf-stalks.

Time		% of stalk soluble in alcohol	% saccharose on T.V.D.M. by		Δ = C.A. − I.	Av. value saccharose %	Hexoses %	Saccharose + hexoses %	In leaf		I.S. C.S.		Pentose %	Pentosan %	Maltose %	$R = \frac{\text{I.S.} + \text{C.S.}}{\text{Matter sol. in alc.}}$ %
			Citric acid	Invertase					Saccharose	Hexoses	In stalk	Leaf				
a.m.	Tops	52·9	3·36	4·14	−0·78	3·75	5·35	9·10	2·51	0·77	1·42	0·31	1·32	10·56	0·00	17·2
	Bottoms	54·3	3·47	3·89	−0·42	3·68	9·11	12·79	„	„	2·48	„	1·20	10·16	„	23·5
noon.	Tops	54·9	4·52	4·26	+0·26	4·39	9·97	14·36	3·11	2·15	2·27	0·69	0·89	10·00	0·00	26·1
	Bottoms	58·3	—	4·12	—	4·12	13·17	17·29	„	„	3·20	„	1·50	9·70	„	29·6
p.m.	Tops	53·6	4·14	3·92	+0·22	4·03	7·89	11·92	2·82	0·96	1·95	0·34	1·20	10·20	0·00	22·2
	Bottoms	57·8	4·03	4·09	−0·06	4·06	10·47	14·51	„	„	2·58	„	1·21	9·65	„	25·1
night.	Tops	50·6	4·12	3·98	+0·14	4·05	6·61	10·66	2·18	0·57	1·63	0·26	1·13	10·47	0·00	21·1
	Bottoms	55·8	—	4·15	—	4·15	8·49	12·64	„	„	2·04	„	1·06	9·82	„	22·6

In Series II the mid-ribs of the leaves were cut out and dealt with separately. The stalks in this series were treated as a whole, and not subdivided into top and bottom halves.

A careful comparison of the data in Tables V, VI, VII and VIII, and Figs. 7 to 10 gives important information as to the translocation of the sugars from the leaf to the root. The following are the principal points disclosed:

1. The proportion of sugars and of matter soluble in alcohol is always far greater in the stalks and mid-ribs than in the leaves at the same picking: thus, for example, in Series I, 12 noon,

Bottoms of stalks contained 17·29 per cent. total sugars,
58·3 per cent. matter soluble in alcohol;
when the *Leaf* contained 5·26 per cent. total sugars,
40·6 per cent. matter soluble in alcohol.

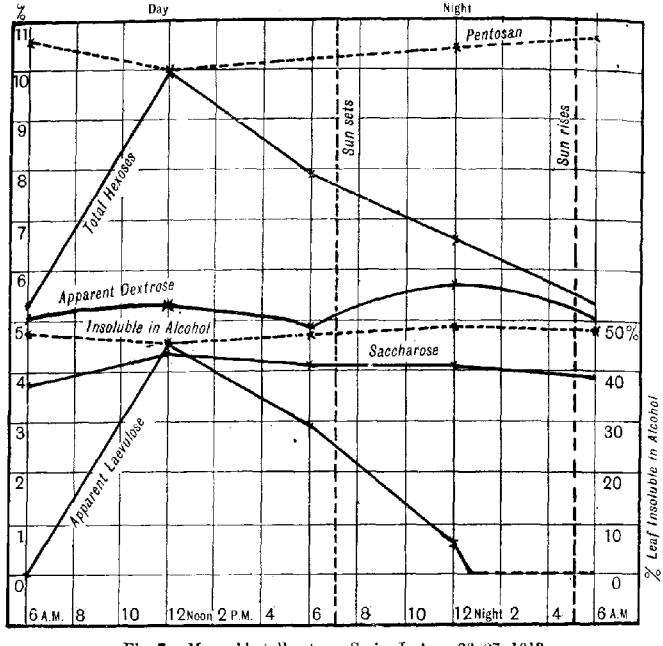

Fig. 7. Mangold stalks, tops, Series I, Aug. 26–27, 1913.

2. As the season advances, a great increase occurs in the proportion of sugars in the stalks at corresponding times of day. Thus, for example, we have:

At 12 noon, August 26th. Total sugars = 15·8 per cent.; matter soluble in alcohol = 56·6 per cent. (average of tops and bottoms).

At 10 a.m., September 10th. Total sugars = 25·3 per cent.; matter soluble in alcohol = 65·4 per cent.

At 11 a.m., October 11th. Total sugars = 30·99 per cent.; matter soluble in alcohol = 63·0 per cent.

In this comparison, in the last two cases samples were not taken at noon, but had they been, the differences would be even greater.

TABLE VI. *Series II.* September 10th–11th, 1912.

Stalks.

Time	% of stalk soluble in alcohol	% saccharose on T.V.D.M. by: Citric acid	% saccharose on T.V.D.M. by: Invertase	Δ = C.A. − I.	Av. value saccharose %	Hexoses %	Saccharose + hexoses %	In leaf: Saccharose	In leaf: Hexoses	I.S./C.S.: In stalk	I.S./C.S.: In leaf	Pentose %	Pentosan %	Maltose %	R = I.S. + C.S. / Matter sol. in alc. %
10 a.m.	65·4	5·25	4·39	+0·86	4·82	20·5	25·32	4·51	5·72	4·25	1·27	1·11	8·01	0·00	38·7
4 p.m.	66·9	5·75	4·78	+0·97	5·26	26·3	31·56	4·38	7·00	5·00	1·60	1·16	7·98	,,	47·2
11 p.m.	65·4	5·18	—	—	5·18	22·4	27·58	6·41	7·10	4·33	1·11	1·06	8·65	,,	42·3
4 a.m.	64·2	5·34	5·10	+0·24	5·22	23·75	28·97	5·62	6·91	4·55	1·23	1·02	8·43	,,	45·1
6 a.m.	64·4	5·25	4·88	+0·37	5·06	26·7	31·76	4·24	6·30	5·27	1·48	1·10	8·84	,,	49·3

TABLE VII. *Series II.* September 10th–11th, 1912.

Mid-ribs.

Time	% of stalk soluble in alcohol	% saccharose on T.V.D.M. by: Citric acid	% saccharose on T.V.D.M. by: Invertase	Δ = C.A. − I.	Av. value saccharose %	Hexoses %	Saccharose + hexoses %	In leaf: Saccharose	In leaf: Hexoses	I.S./C.S.: In stalk	I.S./C.S.: In leaf	Pentose %	Pentosan %	Maltose %	R = I.S. + C.S. / Matter sol. in alc. %
10 a.m.	60·9	6·47	6·22	+0·25	6·35	23·6	29·95	4·51	5·72	3·72	1·27	0·50	10·28	0·00	49·2
4 p.m.	62·8	6·53	5·62	+0·91	6·08	22·6	28·68	4·38	7·00	3·72	1·60	0·98	9·84	,,	45·7
11 p.m.	58·3	6·79	6·58	+0·21	6·68	20·6	27·28	6·41	7·10	3·08	1·11	1·11	10·92	,,	46·8
4 a.m.	64·8	7·42	7·46	−0·04	7·44	19·0	26·44	5·62	6·91	2·55	1·23	1·25	9·52	,,	40·8
6 a.m.	60·3	6·50	6·40	+0·10	6·45	21·4	27·85	4·24	6·30	3·32	1·48	1·07	10·66	,,	46·2

TABLE VIII. *Series III.* October 11th–12th, 1912.

Stalks and Mid-ribs.

Time	% of stalk soluble in alcohol	% saccharose on T.V.D.M. by: Citric acid	% saccharose on T.V.D.M. by: Invertase	Δ = C.A. − I.	Av. value saccharose %	Hexoses %	Saccharose + hexoses %	In leaf: Saccharose	In leaf: Hexoses	I.S./C.S.: In stalk	I.S./C.S.: In leaf	Pentose %	Pentosan %	Maltose %	R = I.S. + C.S. / Matter sol. in alc. %
Stalks:															
11 a.m.	63·0	5·58	5·00	+0·58	5·29	25·7	30·99	7·16	11·62	4·86	1·62	1·01	9·28	0·00	49·2
11 p.m.	61·1	5·54	5·19	+0·35	5·36	21·4	26·76	6·79	9·39	3·99	1·38	1·28	9·51	,,	43·8
Mid-ribs:															
11 a.m.	62·05	6·04													

3. The values of R in the last column, giving the percentage of total sugars in the matter extracted by alcohol, show that as the season advances the sugars form a larger and larger proportion of the total soluble matter which is conveyed by the stalks and mid-ribs. In

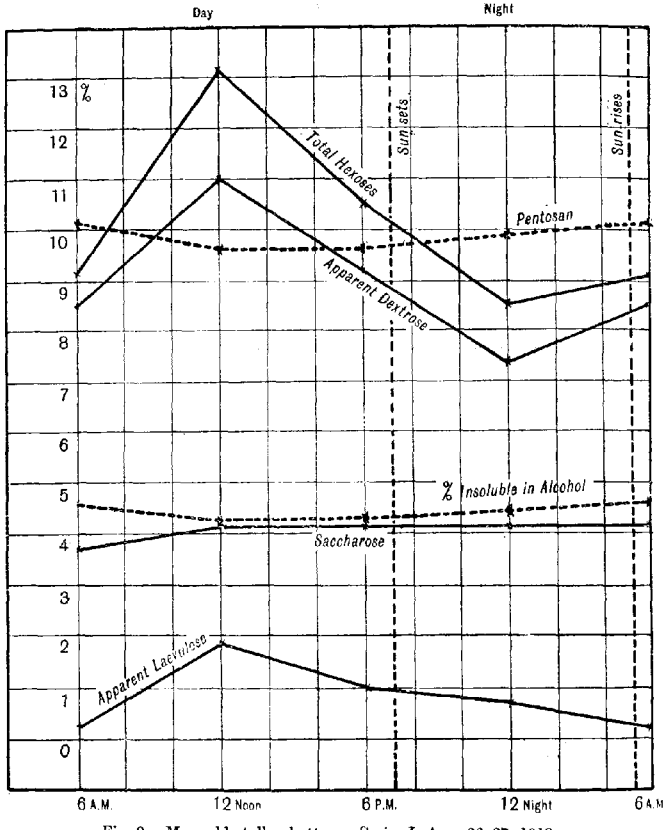

Fig. 8. Mangold stalks, bottoms, Series I, Aug. 26–27, 1913.

Series I, August 26th, the values of R range from 17·2 to 29·6 per cent.; whereas in September and October the sugars formed 40 to 50 per cent. of the total matter soluble in alcohol. The proportion of sugars in this dry matter is at a minimum early in the morning (6 a.m.) and at a

maximum about mid-day, exactly as in the leaves; after reaching the maximum, the sugars (that is the *hexoses*, the saccharose *being practically constant all day*) fall off steadily, almost along a straight line. In the *top* half of the stalks this falling off in the proportion of sugars continues all the night through, but in the bottoms it continues only till midnight, when a slight rise in the proportion of the sugars occurs, owing probably to the rate of inflow from above being greater than the outflow into the roots (see Figs. 7 and 8).

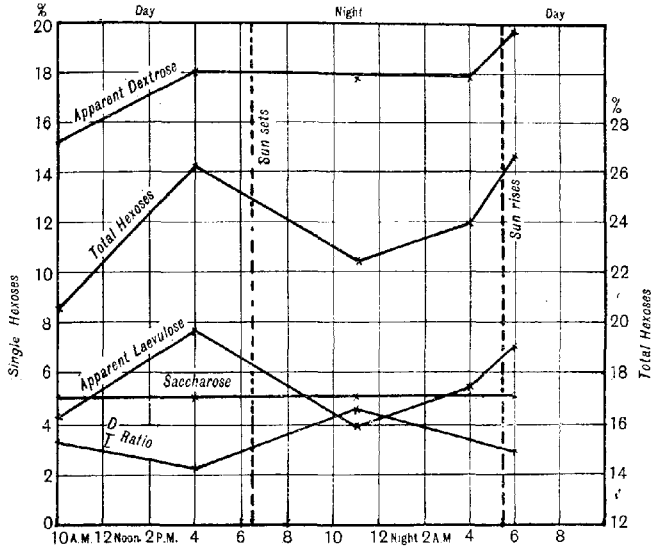

Fig. 9. Sugars in stalks, Mangolds, Series II. Sept. 10-11, 1912.

In the September picking (Series II) (Fig. 9) the saccharose is again practically constant throughout the 24 hours, but the total hexose increases from 10 a.m. to 4 p.m., corresponding with the increase of the leaf sugars which occurs during this interval; at 4 p.m. a falling off of the hexoses occurs in the stalks, which lasts until nearly 11 p.m., and this coincides with the increase of the cane sugar and hexoses in the leaf which occurs at 4 p.m. (see Fig. 5), and was assumed to be due probably to a cessation of translocation from the leaf. It is interesting to note that the large increase in both saccharose and hexoses, which occurs in the leaf between sunset and 2 a.m. (Fig. 5) and was assumed

to be due to the transformation of reserve substances (gums) into sugars, finds its counterpart in the stalk curve (Fig. 9) in the *increase* of the hexoses which starts at night at about 11 p.m. and continues until sunrise; this increase of the sugars in the stalks, occurring moderately rapidly between 11 p.m. and 4 a.m., and then far more rapidly from 4 a.m. to 6 a.m., exactly corresponds with the *rising* portion of the sugar curves in Fig. 5 from 8 p.m. to 2 a.m., followed by the rapid fall from

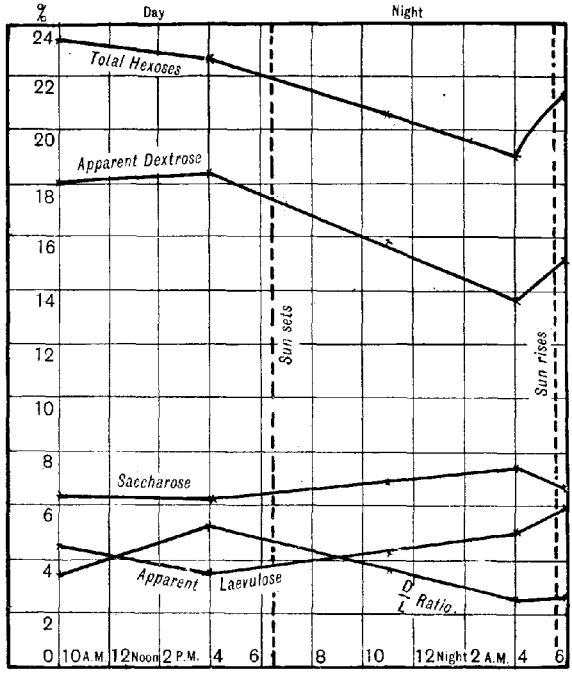

Fig. 10. Mangold mid-ribs, Series II, Sept. 10–11, 1912.

the maxima B and B', which occurs between 2 a.m. and 6 a.m. The rapid fall of the sugars in the leaf is no doubt largely due to their translocation from the leaf to the top of the stalks. It is important to observe (comparing Figs. 9 and 10) that, as might be anticipated if the sugars are formed in the leaf tissue and are thence conveyed to the mid-ribs and stalks, the movement of the sugars in the mid-ribs is always in advance

of the movement in the stalks; thus in Series II, when the sugars are rapidly increasing in the leaf in the morning, the total sugars in the *mid-rib* have a higher value (29·95 per cent. at 10 a.m.) than in the *stalks* (25·32 per cent.), the proportion of sugars to total alcohol-soluble matter being much higher also (49·2 per cent. as compared with 38·7 per cent.). But by 4 p.m. a large proportion of the sugars which were in the mid-rib in the morning have passed into the stalk, so that the numbers *are now reversed* (28·68 per cent. in mid-ribs, 31·56 per cent. in stalks for the sugars; 45·7 and 47·2 per cent. for the proportion of sugars to total alcohol-soluble substances). At 11 p.m. stalks and mid-ribs are practically identical; at 4 a.m. the large accumulation of sugars in the leaf at 2 a.m. is already passing out of the mid-rib and is accumulating in the stalk, thus increasing the proportion of sugars therein, this being shown by the three successive values for total sugars in the stalks, 27·58, 28·97 and 31·76 at 11 p.m., 1 a.m. and 6 a.m. By 10 a.m. a large proportion of the sugar conveyed to the stalk has passed on to the *root*, so that the proportion of sugars falls to its minimum at about this hour.

The problem of translocation is complicated by the fact that several operations are actually occurring simultaneously and the actual analytical data only give the net results of all these; thus sugars are, during the daytime, being formed in the leaves, but at the same time are passing *from* the leaves into the mid-ribs and stalks; it has been shown above that the top and bottom halves of the stalks have very different compositions and the relationship between the sugars in the mid-ribs and stalks and the top and bottom halves of these stalks is again complicated by the fact that the roots are continuously receiving the sugars from the lower part of the stalks, and the tops of the stalks from the mid-ribs. It is interesting in this connection to compare the curves for the total hexoses in the stalks in Series II with those for the mid-ribs in the same series, Figs. 9 and 10. Whereas in the daytime the hexose in the stalks first *increases*, keeping pace with the increased formation in the leaf, and then falls, in the *mid-ribs* the hexose falls continuously throughout the day and night till about 4 a.m. It thus appears that the removal of the sugars from the mid-ribs to the stalks during the day takes place somewhat faster than the sugars pass into the ribs from the leaf tissue. It is seen too that the saccharose content increases somewhat in the mid-ribs during the night, whereas in the stalks it remains practically constant. Both facts are probably due to a common cause, which comes out more clearly from later considerations, that the sugar has to pass from the mesophyll into the veins and

mid-ribs in the form of the less rapidly diffusible sugar saccharose, and is there inverted to reducing sugars; these pass downwards towards the root at a greater velocity than the cane sugar can enter to take their place.

4. The clearest light on the nature of the first sugar formed in the leaf and the changes which occur in translocation is obtained by comparing the values obtained for the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ in the leaves, mid-ribs and top and bottom halves of the stalks at the same hour of the day. Such a comparison shows at once that *the proportion of hexoses to cane sugar is always very small in the leaf as compared with its value in the mid-rib, that it is less in the mid-rib than in the stalk and less in the top half of the stalk (nearest the leaf) than in the bottom half nearer the root.* Thus to take one instance only, on August 26th at 6 a.m. the value of the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ is only 0·307 in the leaf, whereas it is 1·42 in the tops of stalks and 2·48 in the bottoms. It is unfortunate that in Series I no mid-ribs were examined, but on comparing Table VI with Table VII and the values for stalks and mid-ribs in Table VIII, it is seen that the proportion of hexoses to saccharose $\left(\frac{\text{I.S.}}{\text{C.S.}}\right)$ *is always far higher in the stalks than in the mid-ribs at the same time of day.* The stalks in these cases (Series II and III) were the whole stalks, so that the results give merely the average values throughout their length. As seen from Table V, the proportion of hexose in the stalks rapidly increases in passing down the stalks towards the root. A comparison of Table VI with Table V also shows how greatly the ratio of hexoses to saccharose increases in the stalks as the season advances and the storage of sugar in the root becomes more and more the predominating function of the plant; thus for example:

At 6 a.m., August 26th		At 6 a.m., September 10th		
Value of $\frac{\text{I.S.}}{\text{C.S.}}$		Value of $\frac{\text{I.S.}}{\text{C.S.}}$		
In leaf	In stalks (average of tops and bottoms)	In leaf	Mid-ribs	Stalks
0·307	1·95	1·48	3·32	5·27

DISCUSSION OF RESULTS.

What is the primary sugar formed in photosynthesis in the leaf, and in what form are the sugars conveyed to the root?

The results obtained above for the increase of the ratio of hexose to saccharose in passing successively from the leaf to mid-ribs and stalks suggest unmistakably that the cane sugar is formed in the leaf and undergoes a regular and increasing amount of inversion as it passes downwards to the root. Thus at the September picking (4 p.m.), when there was $1\frac{1}{2}$ times as much hexose sugar as saccharose in the leaf, the mid-ribs contained $3\frac{3}{4}$ and the stalks 5 times as much reducing sugar as cane sugar. Thus as an average of the *whole length* of the stalks the cane sugar formed only $\frac{1}{6}$ of the total sugars; bearing in mind the results in Table V, which show how rapidly the proportion of hexose increases in passing from the top to the bottom halves of the stalks, it is clear that the sap in immediate proximity to the root contains probably only $\frac{1}{20}$ or $\frac{1}{50}$ even of its total sugars in the form of cane sugar. This fact taken in conjunction with the relatively high proportion of the cane sugar in the leaf[1] suggests the almost irresistible conclusion that the cane sugar is formed as a primary product in the leaf and is converted into invert sugar for the purpose of rapid transit; this conversion takes place apparently in the veins of the leaf, in the mid-ribs and in the stalks, as the sap travels on its way to the root, and the proportion of sugar inverted steadily increases as the sap nears the root, until in its immediate neighbourhood practically the whole of the sugar is in the form of reducing sugar. The gradual inversion of the cane sugar is probably brought about by the enzyme invertase which is secreted by or distributed on the surface of the sieve tubes, which seem to be the main channels by which the sugar is conveyed to the root (see Peklo [1908]). In accordance with this view is the fact observed by Robertson, Irvine and Dobson [1909][2] that invertase is abundant in the leaf and stem of *Beta vulgaris*, but is absent from the root.

[1] It must be borne in mind that the leaf tissue analysed contained all the smaller veins which could not be removed by the rough method we used; only the large primary mid-ribs were removed. It is probable therefore that the hexoses found in our leaf analyses were mainly present in these veins.

[2] Colin [1915] has independently shown the presence of invertase in the leaves and petioles of the leaf of *Beta vulgaris;* he has established moreover the important fact, that the proportion of invertase is greatest in the leaves, less in the upper part of the stalks and practically nil at the base of the stalk, where it enters the root. In the root itself invertase is entirely absent, as was found to be the case by Robertson, Irvine and Dobson.

The fact that during the *early* stages of growth, when leaf formation is the principal function of the plant and the roots are merely small tap-roots, the cane sugar in the leaf is always in large excess of the hexoses (the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ varying between 0·13 and 0·71) also points strongly to the cane sugar being a primary product and the hexoses as being formed by inversion from this sugar. We show in a separate paper (p. 329) that the proportions of dextrose and laevulose in the mixture of reducing sugars is always nearly that corresponding with this view. In September and October, when the call upon the cane sugar in the leaf is actually greatest for purposes of storage in the root, the ratio of hexoses to saccharose in the leaf rises; it is 0·94–1·59 in September and 1·20–1·95 in October. The relative position of the saccharose and hexose curves thus entirely changes as the function changes; in August the invert sugar curve was much below the cane sugar curve, but in September the positions were reversed. In October, the curve of hexoses was still further above the curve of cane sugar.

The facts we have brought forward as to the translocation of the sugars in the mid-ribs and stalks to our mind outweighs all the other arguments which have hitherto been advanced to show that dextrose and laevulose are precursors of the cane sugar in the leaf. We consider that in spite of the fact that it would be simpler, on theoretical or *a priori* grounds, to consider the hexoses as formed before the more complicated disaccharide, saccharose, the facts we have brought forward are better in accord with Brown and Morris' view that the cane sugar is the primary product of synthesis. It would seem, indeed, that plant leaves in general possess in *the chloroplasts a mechanism for elaborating cane sugar directly from the carbon dioxide of the air*. From the fact that cane sugar is the storage form in the sugar beet and mangold, the argument might be advanced, by those who regard dextrose as the primary product of synthesis, that the presence of cane sugar in the leaf of these plants is exceptional and due to there being here a special mechanism for its production. But we find that even in plants, like the potato (see p. 367), which store starch as a reserve substance, cane sugar is the predominating sugar in the leaf; and even in the grape (*Vitis vinifera*), where dextrose is the storage form, we find that, when special precautions are taken in sampling to prevent the leaf enzymes from inverting the saccharose present, the latter sugar is the principal sugar of the leaf[1].

[1] This is quite contrary to Deleano's statement [1912] that cane sugar is not present in the vine leaf. Deleano's inability to detect saccharose was probably due to insufficient precautions being taken to prevent inversion.

The same is true of the snowdrop, studied by Parkin, which stores starch and inulin.

The relatively high proportion of cane sugar in the mangold leaf during the early stages of growth[1], as well as the experiments recorded by Parkin ([1912], p. 31) showing the enormous increase of the cane sugar which occurs in the leaf of the snowdrop when plants previously kept in darkness for 4 days are exposed to light, support the view that saccharose is actually a direct product of photosynthesis. In Parkin's experiments the cane sugar increased $2\frac{1}{2}$ fold, whilst the hexoses only increased by one-third. It is probable that in the mesophyll of the leaf, saccharose is the *sole* sugar present, and the invert sugar is first formed in the veins or small vessels which serve as conducting channels to the mid-ribs. Our analyses of course could only be made with leaf tissue from which the largest conducting vessels were removed (viz. the mid-ribs), but the tissue actually worked with still contained all the smaller vessels and it was probably in these that the small proportion of reducing sugars found in the early stages of growth was located.

One of the most striking features of the stalks and mid-ribs is that the proportion of saccharose *remains practically constant throughout the whole* 24 *hours*, whilst the hexoses fluctuate between wide limits (see Figs. 7 to 10). Thus in Series I, for example, the cane sugar in the tops of the stalks varied only from 3·75 to 4·39 per cent., whilst the hexoses had a range of from 5·35 to 13·17 per cent. Moreover, whilst the percentages of reducing sugars in the top and bottom halves are widely different at any one time, the bottom half always being the richer, the proportion of saccharose in the two halves is practically the same. Thus, for example, at noon (Table V) when the invert sugar in the top half was 9·97 per cent. and in the bottom half 13·17 per cent., the cane sugar in the top half was 4·39 and in the bottom half 4·12 per cent. As the season advances the proportion of cane sugar at corresponding times of day changes but little, whereas the hexoses increase enormously. For example, we may take the following:

[1] The predominance of cane sugar in the leaf in the early stages of growth points to the rate of production of this sugar exceeding the rate of its hydrolysis by invertase; the fact that during the morning the hexoses (see Fig. 4) increase faster than the cane sugar points to the hydrolytic effect of the enzyme increasing more rapidly with rise of temperature (or an actual increase in the amount of enzyme occurring) than the synthetic effect producing the saccharose. The parallelism of the curve showing the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ with the temperature curve in both Series I and II is best explained in this way.

	August 26th, noon (average tops and bottoms)	Sept. 10th, 10 a.m.	Oct. 11th, 11 a.m.
Saccharose	4·25 %	4·82 %	5·29 %
Hexoses	11·57	20·5	25·7

It is a striking fact (compare Table V with Table VI and the data for mid-ribs and stalks in Table VIII) that the proportion of saccharose in the mid-ribs (which are nearer the leaf tissue) is slightly higher and somewhat less constant than in the stalks. For example, in Series II:

In *stalks*, saccharose varies from 4·82–5·26 per cent.; hexoses from 20·5–26·7 per cent.

In *mid-ribs*, saccharose varies from 6·08–7·44 per cent.; hexoses from 19·0–23·6 per cent.

On passing from the stalks, through the mid-ribs to the leaves, the range of variation of the saccharose during the day *increases*, but even in the leaves the variation of cane sugar *during the daytime* is far less marked than that of the hexoses. Parkin also observed in the snowdrop a similar phenomenon (*loc. cit.*, p. 28) as regards the seasonal variation, but the fluctuation of the hexoses between morning and evening was less marked than that of the saccharose (*loc. cit.*, p. 29). This was probably due to the fact that he took only two samples in the day, one at a time when the sugars were increasing, the other when they were falling. A similar false conclusion as to the approximate constancy of the hexoses and wide variation of the saccharose would be formed if samples had been taken in Series III (Fig. 6) at 9 a.m. and 3 p.m. only, but the general shape of the curves in Series I and II shows that during the *period of daylight* the hexose is the more variable even in the leaf.

The great variation during the day of the reducing sugars and the practical constancy of the cane sugar in the mid-ribs and stalks, and the fact that the proportion of cane sugar in the top and bottom halves of the stalks is practically the same, point to the relatively rapid movement or formation of the hexoses in the mid-ribs and stalks. In the case of the snowdrop, Parkin (*loc. cit.*, p. 25) found that the long, narrow *leaves* contained a far larger proportion of hexose to cane sugar in the upper parts than in the lower parts, especially when the plants were grown in clumps so that the lower parts were shaded; in this case, the lower halves of the leaves functioned mainly as stalks or media of translocation not as true assimilating leaves, the results presenting an exact parallel with those obtained in the case of the mangold stalks. Parkin also found that the *colourless* part of the snowdrop leaf which is enclosed by the membranous sheath is very rich in sugar—30 to 40 per cent. of its

dry weight. On analogy with our results these lower portions of leaf correspond with the extreme lower parts of the mangold leaf-stalks, and would probably, if an analysis were made, be found to contain practically the whole of the sugar in the form of reducing sugar.

In the potato (see pp. 367–373), where saccharose is the predominating sugar in the leaf, the proportion of hexose in the stalks bearing the leaflets is far higher than in these leaflets (5 to 30 fold) whilst the saccharose is very nearly the same in both.

	Hexoses in		Saccharose in		Ratio $\frac{\text{I.S.}}{\text{C.S.}}$ in	
	Stalk	Leaflets	Stalk	Leaflets	Stalk	Leaflets
Minimum ...	4·63	0·15	2·65	1·76	0·10	1·54
Maximum ...	5·63	1·27	3·58	3·66	0·44	1·77

It would seem, therefore, that *in all plants of which a systematic examination has been made (mangold, sugar beet, potato, snowdrop, grape vine, dahlia, etc.) saccharose is formed directly in the mesophyll of the leaf, whence it passes into the veins, mid-ribs and stalks, undergoing more and more complete inversion in its passage.* The regulating mechanism is apparently such that a nearly constant concentration of cane sugar is maintained throughout the day and throughout the season in the mid-ribs and stalks, whilst the reducing sugars vary within very wide limits.

At first sight it would appear to be a clumsy and unnecessary contrivance for plants such as the mangold and sugar beet to form cane sugar in the leaf, then to transform it completely into hexoses in the stalks only to have to reconvert it back again into cane sugar in the roots; it would seem to be a simpler arrangement for the cane sugar to travel as such to the roots. As will be seen from the historical introduction given on pp. 255–262, most workers in this field have assumed this to be the case. It is a striking fact that even Girard's [1884] data show an enormous preponderance of the hexoses over the saccharose in the leaf-stalks of the sugar-beet (the hexoses being 5 to 10 times the cane sugar, which generally was small); but he was apparently so struck by the novel observation of the relatively large proportion of cane sugar in the leaf, that he quite ignored the significance of the stalk analyses, concluding that the saccharose passed *tout formé* from the leaf to the root.

It is probable however that the actual mechanism of storage adopted possesses certain well-defined advantages; in the first place, if the sugars travel by simple diffusion, as has frequently been assumed, the rate of

diffusion of the reducing sugars would be four times that of the cane sugar; moreover, as was emphasised by Brasse [1886], if the storage of cane sugar is accomplished by a direct wandering of this sugar as such from the leaf to the root, it would be in direct defiance of the ordinary laws of diffusion, as motion would occur from a place of low concentration to one of high concentration. If, however, the sugar is translocated as hexose and is immediately transformed in the root into saccharose, these objections no longer hold and a continuous stream of sugar could be maintained. It is probable, too, that the actual mechanism adopted serves to keep the cane sugar, once it is formed in the root, from getting out; de Vries' [1879] well-known experiment may be recalled, in which he showed that strips of beet-root could be soaked in water for 14 days without the presence of sugar being detected in the surrounding water. Gutzeit [1911] has also recently emphasised the impermeability to saccharose of the protoplasm of the cell-walls of the beet-root, pointing out that in the ordinary process of manufacture of sugar from the beet it is necessary to use *hot* water first, to kill the protoplasm, before the sugar can be extracted. Brasse [1886] showed that the same effect could be produced by chloroform. If the protoplasm is permeable to the hexoses and not to the saccharose a simple mechanism would exist by which the root could store up sugar without any possibility of loss by back-diffusion, and an explanation would be given of the fact that although the *concentration* of sugar in the root may be diminished after heavy rains by the inflow of water, the actual *total quantity* of sugar in the root steadily increases throughout the season's growth and never shows any falling off (Vivien [1913]). It has been generally assumed that, in the *second* year's growth of the beet, when the cane sugar is utilised to form new shoots, the saccharose is first inverted by invertase in the root and is conveyed in the form of invert sugar to the growing points. But according to Colin [1915] invertase is not found in the *root* even in the second period of growth, when the seed-bearing plant is beginning to form; the saccharose is held to undergo inversion in the stalks and leaves of the new plant.

It still remains to consider the means by which the hexoses which are carried into the root are transformed into saccharose. The complete absence of invertase from the root militates against the view adopted by Robertson, Irvine and Dobson [1909] that the cane sugar is formed by a process of reversible zymo-hydrolysis, in which the invertase acts as a synthetic agent. These authors recognised this difficulty and were driven to assume that saccharose is formed, not

in the root, but in the organs containing invertase—the leaves and stalks—and translocated as such. Our results prove that the sugars approaching the neighbourhood of the root become richer and richer in hexoses, and, as we shall shortly show, it is in the form of hexoses that the sugars actually enter the root. With regard to the theory that the cane sugar is formed by reversible enzyme action, the experiments quoted by Robertson, Irvine and Dobson to show a synthetic action of the enzyme-sludge prepared from parts of the sugar-beet are by no means conclusive; even if such synthetic action occurred (and its amount seemed to be exceedingly small) it is not shown that the synthetic action was due to invertase or that it was reversible. In all ordinary concentrations such as would be met with in the plant cells, invertase acts practically completely in the one direction only—that of hydrolysis. It would seem indeed that the root of the sugar beet or mangold possesses some special mechanism for synthesising cane sugar—some special enzyme such as the "saccharogenic enzyme" of Barbet [1896]. But there is as yet little direct evidence available in favour of such a theory.

That the reducing sugars conveyed by the stalks actually enter the root is shown by the recent analyses of Colin [1914], who states that when the root is exceedingly small (*souches filiformes*) the reducing sugars form one-fifth of the total sugars in the root. The *proportion* of reducing sugar naturally falls as the season advances, because the accumulation of the cane sugar stored diminishes the *relative* proportion of reducing sugar. But there is little doubt from the analyses we give in Table V that the reducing sugars, which more and more predominate in the stalks as the root is approached, actually enter the root as such throughout the season. Pellet [1914, 2], at the congress of sugar chemists in 1914, criticised the work of earlier workers, such as Girard, and pointed out that their failure to detect reducing sugars in the root was owing to their having used insufficiently delicate methods of analysis; he stated that reducing sugars are always present in the root in amounts varying from 0·05 to 0·30 per cent., depending on the meteorological conditions. When the hexoses are being rapidly formed and enter the root at a rate which is in excess of the ability of the "saccharifying power" of the root to cope with, the reducing sugars accumulate for a short time, and high values such as 0·3 per cent. are obtained. On other days, when the rate of production of the hexoses is less, the "saccharifying power" of the root is able immediately to transform the whole of the hexoses into saccharose, and lower values such as 0·02 to 0·03 per cent. are found.

Even in the typical saccharose-forming plant the sugar cane, M. Pellet informs us (private communication), reducing sugars are invariably present. In the early stages of growth (first 5 or 6 months) the cane may contain 3 per cent. of saccharose and 3 per cent. of hexoses; later the proportion becomes 5 per cent. of saccharose and 2 per cent. of hexoses, then 7 per cent. of saccharose and 1 per cent. of hexoses until finally the cane contains 12, 13 or even 16 per cent. of saccharose and only 0·6, 0·5, 0·2, or even 0·1 per cent. of reducing sugars[1]. The quantity of hexoses in the cane differs in different parts, increasing as one passes from the lower to the upper part of the stem. This, we consider, points to a steady influx of reducing sugars from the upper parts, followed by transformation and storage in the lower parts of the cane, which in this plant fulfil the functions of the root in the sugar beet and mangold.

Finally it will be well to emphasise the difference that exists between our views and those recently expressed by Pellet [1913] and by Colin [1914]. Pellet holds that saccharose, dextrose and laevulose are formed *simultaneously* (*tout à la fois*) in the leaves and that *all* these sugars pass into the root, which possesses the property of transforming the hexoses into saccharose. Colin expresses practically the same view—"la racine reçoive à la fois du saccharose qui s'emmagasine et du réducteur qui est polymerisé." We are quite at one with these workers that saccharose, dextrose and laevulose are *present* at the same time, but we consider that the cane sugar is probably first formed *alone* in the mesophyll of the leaf, that it is transformed into invert sugar by invertase in the veins and mid-ribs and finally more and more completely in its progress through the sieve-tubes of the stalks. It enters the root entirely in the form of reducing sugars and is therein reconverted into saccharose. Once in this form the sugar cannot escape, until it is put under contribution at the commencement of the following season's growth, for the building up of the new shoot.

Strakosch's views. Strakosch [1907] has put forward the view, based on micro-chemical tests, that in the mesophyll of the leaf only one sugar is present, namely, dextrose; laevulose is said first to occur in the small veins of the leaf and saccharose is formed as a final product in the veins and mid-ribs. It is in the form of saccharose that the sugar travels to the roots. Strakosch employed Grafe's [1905] micro-chemical test for laevulose, based on the use of methylphenylhydrazine; whether

[1] Pellet [1914, 1] shows that the reducing sugars found in the molasses of cane sugar manufacture represent the original hexoses of the juice and are not produced by inversion during the manufacturing operations.

this test is at all characteristic of ketoses is still an open question. Strakosch and Neuberg [1904] consider that it is, but Ofner [1904 and 1905] and Ost [1905] regard it as unsatisfactory in presence of dextrose. With regard to Senft's [1904] test for dextrose which Strakosch relied upon it is perhaps sufficient to point out that Strakosch himself admits that "die Unterscheidung von Rohrzucker und Glucose nach dem Senftschen Verfahren ist an die richtige Schätzung des untersuchenden Auges gebunden[1]." Absolutely in contradiction with Strakosch's views, but in accord with our own, are the earlier micro-chemical observations of de Vries [1897], who found in the chlorophyll-containing cells of the sugar beet *no* reducing sugars at all, in the general tissue of the vascular bundles only small quantities, but in the larger vessels and veins increasing quantities of hexoses.

Strakosch relies almost entirely on his micro-chemical tests. He quotes only two quantitative estimations of the sugars; in one, an extract of mesophyll tissue was found to contain 0·15 per cent. of hexoses and 0·026 per cent. of saccharose; in the other, an extract of vein tissue contained 0·12 per cent. of hexoses and 0·54 per cent. of saccharose. No details are given as to the preparation of these extracts, nor of the means taken to prevent enzyme changes. The results given absolutely contradict those obtained by Kayser [1883], Girard [1884], Ruhland [1911], Parkin [1912], Colin [1914] and ourselves,

[1] Since the above was written Mangham (*Annals Bot.* 1915, **29**, 369) has published some observations on the osazone method of locating sugars in plant tissues. As a result of his experiments he considers (p. 377) that any attempt to distinguish saccharose qualitatively in presence of its hexose constituents by Senft's method cannot give trustworthy results. Senft was led to attach too much importance to this method from the results obtained with 50 per cent. sugar solutions, and neglected to check them with weaker solutions comparable in concentration with the contents of plant cells. Mangham, therefore, like ourselves, considers that Strakosch's technique was unreliable and "only those of his conclusions with regard to cane sugar which were founded on evidence other than that derived in the above manner can be regarded as at all trustworthy." This would leave very little of the structure raised by Strakosch still standing.

It is difficult to understand Mangham's view that it is possible to discriminate between dextrose and laevulose by means of the osazone test, seeing that both sugars (as well as mannose) yield identically the same osazone: Mangham seems to regard the osazones from dextrose and laevulose as distinct substances.

In the writer's opinion little reliance can be placed on a micro-chemical osazone test as a means of identifying maltose in plant tissues, owing to the presence of large quantities of other sugars. Our quantitative analyses (some 500 in all) have in no single instance disclosed the presence of even traces of maltose in the leaves or conducting systems of plants. In work of this kind, micro-chemical tests as a means of distinguishing individual sugars should be avoided and only quantitative methods adopted. Otherwise contradictory and uncertain results are inevitable. [Note added, Dec. 9, 1915.] W. A. D.

which show that in passing from the leaf tissue to mid-ribs and stalks, the proportion of hexoses to saccharose progressively and rapidly increases. If Strakosch's views were correct we should expect to find the hexoses in excess in the leaf tissue and the cane sugar steadily increasing on the way to the root. Actually the reverse is true. In the earliest stages of growth, saccharose predominates in the leaf and even in the later stages of growth *when the hexoses are in excess in the leaf, the proportion of cane sugar to reducing sugars is at its maximum in the leaf tissue and falls steadily in passing from the leaf to mid-ribs, and from mid-ribs to stalks.* If Strakosch's ideas were correct it is surprising to find, as the season advances and the importance of the storage function increases, the *proportion* of the reducing sugars to cane sugar in the stalks actually *increasing*; thus:

	Stalks, Aug. 26th–27th (average top and bottom halves)	Stalks, Sept. 10th–11th
Ratio $\frac{\text{I.S.}}{\text{C.S.}}$ ...	ranges from 1·8 to 2·7	4·25–5·27
Hexoses ...	5·35–13·1 %	20·5–26·7 %

The following comparisons of the ratio of hexoses to saccharose for leaf, mid-ribs and stalks are incomprehensible on the basis of Strakosch's views.

	Leaf	Mid-ribs	Stalks
August 26th–27th ...	0·26–0·69	—	1·8 –2·7
September 10th–11th	1·11–1·60	2·55–3·72	4·25–5·27
October 11th–12th ...	1·14–1·93	2·75–3·24	3·99–4·86

We are aware that against the view that cane sugar is a primary product may be urged the fact that in Series I and II of our results the hexoses appear to increase after sunrise faster than the saccharose, so that they seem to be more responsive to the stimulus of light than the saccharose. But this is probably due to the fact that each molecule of saccharose gives rise on inversion to two molecules of hexose; when the separate proportions of dextrose and laevulose are considered (see following communication) they appear to follow the proportion of cane sugar more closely. The actual values found for cane sugar represent merely the excess of cane sugar formed over that inverted to hexoses; as we have already pointed out the proportion of hexoses to saccharose $\left(\frac{\text{I.S.}}{\text{C.S.}}\right)$ *during the daytime* (that is the period of photosynthetical action) follows very closely the temperature curve, as if the rate of inversion of

the cane sugar increased with the temperature proportionately faster than the rate of formation of cane sugar itself.

We are also aware that it is possible to explain the predominance of saccharose in the early stages of growth (Series I), regarding the hexoses as primary products, by assuming that as the root is insufficiently developed to deal with them, they are transformed into the storage form, saccharose, in the leaf itself, so as to relieve the osmotic pressure. The formation of starch in the very early stages of growth, contrasted with its entire absence later on, is a similar phenomenon, having as its object the diminution of the excess sugar formation. But although this hypothesis is a possible one, it appears that the whole body of facts we have recorded, especially the data regarding translocation, find a better explanation in the view that the cane sugar is a primary product and gives rise to the hexoses by inversion than by assuming the hexoses to be primary products in the mesophyll and the saccharose to be formed from them.

SUMMARY.

1. The formation and translocation of the sugars in the mangold have been studied under actual conditions of growth, in which translocation was normal.

2. Starch is entirely absent from the leaf after the very earliest stages of growth. As soon as the root begins to develop so that the sugars formed in the leaf can be translocated to it, starch disappears almost entirely from the leaf. Maltose is entirely absent from leaf, mid-ribs and stalks at all stages of growth and at all times of night and day.

3. During the early stages of growth of the mangold, when leaf formation is the principal function, saccharose is present in the leaf tissue in excess of the hexoses. Later in the season, when sugar is being stored in the root, the reverse is true, hexoses largely predominating in the leaf.

4. In the mid-ribs and stalks the hexoses always predominate greatly over the saccharose and vary widely in amount during the day and night, and throughout the season, whilst the saccharose remains practically constant. In passing from leaves to mid-ribs, from mid-ribs to the tops of stalks and from the tops of stalks to the bottoms, the ratio of hexoses to saccharose steadily and rapidly increases. As the season advances the predominance of the hexoses in leaf, mid-ribs and stalks becomes more and more marked.

5. The proportion of saccharose in the leaf tissue follows the temperature curve closely during the daytime; the proportion of hexoses increases faster than the temperature, in such a way that the curve showing the ratio of hexoses to saccharose is itself practically parallel to the temperature curve.

6. The facts brought forward can apparently be best explained by Brown and Morris' view that saccharose is the primary sugar formed in the mesophyll of the leaf under the influence of the chlorophyll; it is transformed into hexoses for the purpose of translocation. This transformation occurs in the veins, mid-ribs and stalks, the proportion of hexoses increasing more and more as the root is approached. The sugar enters the root as hexose and is therein reconverted into saccharose; once in this form the saccharose is not able to leave the root until it is put under contribution for the second season's growth.

7. These views are in accord with de Vries' micro-chemical observations as to the nature of the sugars in the different tissues but entirely in contradiction with those of Strakosch, which are considered to rest on no secure foundation.

8. They also agree with Parkin's results with the snowdrop, with Pellet's analyses of the sugar cane, with Colin's results with the sugar beet and our own observations with other plants such as the vine (*Vitis vinifera*), potato, dahlia, etc., which store their carbohydrates in other forms (dextrose, starch and inulin).

9. As regards the mechanism by which saccharose is synthesised from the hexoses, it is improbable that this change is effected by invertase by a process of reversible zymo-hydrolysis. The entire absence of invertase from the root is against this view.

10. Pentoses form only a small proportion of the total sugars in the tissues; they are probably formed from the hexoses and appear to be precursors of the pentosans.

APPENDIX.

A. EXAMPLE SHOWING METHOD OF CALCULATING RESULTS.

Mangold Leaves. October 11th, 1912, 11 p.m.

The alcoholic extract of the leaf material was evaporated *in vacuo* and made up to 500 cc.

20 cc. of the 500 cc. evaporated and dried *in vacuo* at 110° in the apparatus, Fig. 2, gave (*a*) 2·5833 grms.

(*b*) 2·5872 „

Average = 2·5852 grms. = **64·63** grms. in 500 cc.

The leaf residue left after extracting the sugars, etc., weighed 75·31 grms. after drying at 100°. This still contained 6·84 % moisture, as determined by heating 10 grms. *in vacuo* at 110° (see p. 269).

∴ Weight of vacuum-dried matter in the 75·31 grms. = 75·31 × 0·9316 = **70·16** grms.

Total vacuum-dried matter in leaf sample = 64·63 + 70·16 = **134·79** grms.

$$\frac{\text{Matter extracted by alcohol}}{\text{Total vacuum-dried matter}} = \frac{64{\cdot}63}{134{\cdot}79} \times 100 = \mathbf{47{\cdot}9}\ \%.$$

FOR SUGARS.

440 cc. of the 500 cc. were precipitated by basic lead acetate (265 cc.), filtered and washed to 2 litres = *Solution A*.

300 cc. of *A* deprived of lead by solid sodium carbonate and made to 500 cc. = *Solution B*.

For reducing sugars.

25 cc. of *B* (= 15 cc. *A*) gave 0·2210 grm. CuO[1].

Rotation of Solution *B* in 200 mm. tube at 20° (Na flame) = + 0·274°.

For cane sugar.

50 cc. of Solution *B* were inverted by either citric acid or invertase. After inversion and neutralisation, made to 100 cc. = *Solution C* (or *C'*)

(*a*) *Citric acid.*

50 cc. of *C* (= 25 cc. *B* = 15 cc. *A*) gave 0·3710 grm. CuO.

Solution *C* gave rotation = − 0·064° in 200 mm. tube at 20°.

[1] In all cases the value of the cupric reducing power given is the average of two or three closely concordant results. The separate determinations always agreed to within 1 to 2 mgs. of CuO.

(*b*) *Invertase.*

50 cc. of *C'* (= 15 cc. *A*) gave 0·3706 grm. CuO.

Solution *C'* gave rotation = − 0·071° in 200 mm. tube at 20°.

Maltose.

300 cc. of Solution *A* deprived of lead by hydrogen sulphide were diluted to 500 cc. = *Solution B'*.

Three 50 cc. portions of Solution *B'* were fermented by *S. exiguus*, *S. marxianus* and *S. anomalus*. After treatment with 5 cc. of alumina cream each solution was made to 100 cc.; 50 cc. of the filtrate (= 15 cc. *A*) gave 0·0134 grm. CuO (average of the three).

Two 50 cc. portions were fermented similarly with distillery yeast. 50 cc. of the final solution (= 15 cc. *A*) gave 0·0127 grm. CuO (average of the two).

Pentoses.

50 cc. *A* gave 0·0149 grm. phloroglucide.

$$\text{Pentose} = (0{\cdot}0149 + 0{\cdot}0052) \times 1{\cdot}017 \times \frac{2000}{50} \times \frac{500}{440} \times \frac{100}{134{\cdot}79} = \mathbf{0{\cdot}68}\ \%$$

on the T.V.D.M.

Calculation of Results.

Saccharose. (*a*) *Citric acid.*

From reduction. Increase of reduction in 25 cc. *B* (= 15 cc. *A*) caused by inversion

$$= 0{\cdot}3710 - 0{\cdot}2210 = 0{\cdot}1500 \text{ grm. CuO.}$$

This corresponds, using the factor for 0·3710 grm. of CuO in Brown, Morris and Millar's Tables, to $\frac{0{\cdot}1500}{2{\cdot}354}$ grms. of invert sugar.

% of saccharose on T.V.D.M. of leaf

$$= \frac{0{\cdot}1500}{2{\cdot}354} \times 0{\cdot}95 \times \frac{2000}{15} \times \frac{500}{440} \times \frac{100}{134{\cdot}79} = \mathbf{6{\cdot}80}\ \%.$$

(*b*) *Invertase.* Increase of reduction on inversion of 15 cc. *A*

$$= 0{\cdot}3706 - 0{\cdot}2210 = 0{\cdot}1496 \text{ grm. CuO.}$$

$$\therefore\ \%\ \text{of saccharose (as in } (a)) = \mathbf{6{\cdot}78}\ \%.$$

From Polarisation data. (*a*) *Citric acid.*

Change of rotation of *B* in 200 mm. tube caused by inversion

$$= 0{\cdot}274^\circ + (2 \times 0{\cdot}064^\circ) = 0{\cdot}402^\circ.$$

$$\% \text{ of saccharose} = \frac{0{\cdot}402}{1{\cdot}744^*} \times \frac{500}{100} \times \frac{2000}{300} \times \frac{500}{440} \times \frac{100}{134{\cdot}79} = \mathbf{6{\cdot}48}\ \%.$$

(*b*) *Invertase.* Change of rotation of *B*

$$-0{\cdot}274^\circ + (2 \times 0{\cdot}071^\circ) = 0{\cdot}416^\circ.$$

$$\therefore\ \% \text{ of saccharose (as in } (a)) = \mathbf{6{\cdot}70}\ \%.$$

Apparent Dextrose and Laevulose.

Reduction of 25 cc. *B* (= 15 cc. *A*) = 0·2210 grm.

Reduction due to pentoses in 25 cc. *B*

$$= \frac{15}{50} \times 0{\cdot}0201 \times 1{\cdot}017 \times 2{\cdot}65\dagger = 0{\cdot}0163 \text{ CuO}.$$

$$\therefore \text{ Reduction due to hexoses} = \mathbf{0{\cdot}2047} \text{ grm. CuO}.$$

Grms. pentose in 100 cc. *B*

$$= 4 \times \frac{15}{50} \times 0{\cdot}0201 \times 1{\cdot}017 = 0{\cdot}0245 \text{ grm}.$$

Rotation of pentose in *B* in 200 mm. tube.

If *arabinose* = $102{\cdot}2 \times 200 \times 0{\cdot}0245 = \mathbf{0{\cdot}050}^\circ$.

If *xylose* = $18{\cdot}8 \times 200 \times 0{\cdot}0245 = \mathbf{0{\cdot}009}^\circ$.

Now the concentration of saccharose in 100 cc. *B*

$$= \frac{6{\cdot}79}{100} \times 134{\cdot}79 \times \frac{440}{500} \times \frac{3}{5} \times \frac{1}{20} = 0{\cdot}2417 \text{ grm}.$$

Rotation due to saccharose in *B* (200 mm. tube)

$$= \frac{66{\cdot}44 \times 200 \times 0{\cdot}2417}{10^4} = \mathbf{0{\cdot}321}^\circ.$$

$\therefore$ Rotation due to hexoses in *B*,

calculating pentoses as arabinose = $0{\cdot}274 - 0{\cdot}050 - 0{\cdot}321^\circ = -\mathbf{0{\cdot}097}^\circ$,

,, ,, ,, xylose = $0{\cdot}274 - 0{\cdot}009 - 0{\cdot}321^\circ = -\mathbf{0{\cdot}056}^\circ$.

* The factor 1·744 represents the change of rotation in a 200 mm. tube caused by the inversion of a 1 per cent. solution of saccharose.

† Assuming the pentoses to be a mixture of equal quantities of arabinose and xylose and using the tables given by Daish [1914] for the reducing power of the pentoses. The value 0·0163 found is slightly higher than the residual reduction 0·0130 after fermentation, which is due to the pentoses.

Proportions of Dextrose and Laevulose.

(A) *Pentoses as arabinose.* If x = grms. of dextrose in 25 cc. B,
y = grms. of laevulose in 25 cc. B,

we have[1] $4{\cdot}22x - 7{\cdot}36y = -0{\cdot}097°$,
$2{\cdot}56x + 2{\cdot}34y = 0{\cdot}2047$.

Solving for x and y,

$x = 0{\cdot}04457$ grm. dextrose in 25 cc. B (= 15 cc. A),
$y = 0{\cdot}03872$ grm. laevulose in 25 cc. B (= 15 cc. A).

$$\therefore \text{ Dextrose in T.V.D.M.} = 0{\cdot}04457 \times \frac{2000}{15} \times \frac{500}{440} \times \frac{100}{134{\cdot}79} = \mathbf{5{\cdot}01}\ \%.$$

$$\text{Laevulose in T.V.D.M.} = 0{\cdot}03872 \times \frac{2000}{15} \times \frac{500}{440} \times \frac{100}{134{\cdot}79} = \mathbf{4{\cdot}35}\ \%.$$

$$\therefore \text{ D} + \text{L} = \mathbf{9{\cdot}36}\ \%.$$

$$\frac{\text{D}}{\text{L}} = \frac{5{\cdot}01}{4{\cdot}35} = \mathbf{1{\cdot}151}.$$

(B) *Pentoses as xylose.* $4{\cdot}22x - 7{\cdot}36y = -0{\cdot}056°$,
$2{\cdot}56x + 2{\cdot}34y = 0{\cdot}2047$.

Hence as in (A), % of dextrose = **5·39** %
% of laevulose = **3·94** %
D + L = **9·33** %

$$\frac{\text{D}}{\text{L}} = \frac{5{\cdot}39}{3{\cdot}94} = \mathbf{1{\cdot}37}.$$

Calculating Reducing Sugars as Invert Sugar.

$$\text{Invert sugar in 25 cc. } B = \frac{0{\cdot}2047}{2{\cdot}445} = 0{\cdot}08351 \text{ grm.}$$

$$= 0{\cdot}08351 \times \frac{2000}{15} \times \frac{500}{440} \times \frac{100}{134{\cdot}79} = \mathbf{9{\cdot}39}\ \% \text{ on T.V.D.M.}$$

If the rotation in B were due to invert sugar, in 200 mm. tube at 20°, rotation

$$= \frac{0{\cdot}08351 \times 4 \times 200 \times -19{\cdot}6}{10} = \mathbf{-0{\cdot}131°}.$$

[1] The constants 4·22 and 7·36 for dextrose and laevulose are calculated from the specific rotatory powers $[\alpha]_D^{20°} = 52{\cdot}7$ and $[\alpha]_D^{20°} = -92{\cdot}0°$ for 1 per cent. solutions of the sugars. The constants 2·56 and 2·34, corresponding with the 0·2210 grm. CuO actually weighed, are taken from Brown, Morris and Millar's Tables for dextrose and laevulose.

Instead of actually observed, pentoses as arabinose = $-$ **0·094°**,
and actually observed, pentoses as xylose = $-$ **0·053°**.

Maltose. Reduction corresponding with 15 cc. *A*.

After fermentation with maltase-free yeasts = 0·0134 grm. CuO
After fermentation with distillery yeast = 0·0127
Δ = 0·0007 grm. CuO

The difference between the two sets of fermentations is within the experimental error of the method.

$\therefore$ Maltose = **0·00** %.

Starch. Exp. 1. 10·449 grms. of oven-dried leaf matter after extracting sugars gave, on drying *in vacuo* at 110°, 9·7355 grms.

$\therefore$ Moisture = **6·82** %.

The leaf material was heated with 200 cc. of water in boiling water for 15 minutes and, after cooling, treated with 0·1 grm. taka-diastase at 38° for 24 hours. After boiling to destroy the enzymes, the leaf material was filtered on a Buchner funnel and washed with water until the washings amounted to about 450 cc. The filtrate was transferred to a 500 cc. measuring flask and exactly the necessary quantity of basic lead acetate solution (8·5 cc.) added to precipitate the tannins, etc. The solution was then, without filtering, made up to 500 cc. at 15°. After filtering, 100 cc. of the filtrate were treated with solid sodium carbonate to precipitate the lead present and made up to 110 cc. After filtering, 50 cc. were used to estimate the reducing power.

Weight of CuO = 0·0000 grms.
Polarisation in 400 mm. tube = $-$ 0·053°.

The entire absence of reducing power shows the absence of maltose and dextrose and hence of starch in the original material. The laevo-rotation is due to the gummy matter of the leaf which is not entirely precipitated by the basic lead acetate, owing to the slight solubility of its lead compound (Davis and Daish [1914]).

Exp. 2. Gave Moisture = 6·86 %,
Starch = 0·00 %.

B. EXPERIMENTAL DATA.

Series I. Mangold Leaves. August 26th–27th, 1913.

The alcoholic extract was evaporated *in vacuo* and made up to 500 cc. 440 cc. of this solution were treated with basic lead acetate

and the filtrate and washings deprived of lead by adding solid sodium carbonate: the solution was then diluted to 2000 cc. = *A*.

Time	Dry matter sol. in alcohol (vacuum-dried), grms.	Dry matter insol. in alcohol (vacuum-dried), grms.	Total vacuum-dried matter (T.V.D.M.), grms.	Direct reducing power of 25 cc. *A*, grms. CuO	Polarisation of *A* in 200 mm. tube, α_D^{20}	Invertase inversion: Reduction of 25 cc. *A* after inversion, grms. CuO	Invertase inversion: α_D after inversion in 200 mm. tube*	Citric inversion: Reduction of 25 cc. *A* after inversion, grms. CuO	Citric inversion: α_D after inversion in 200 mm. tube*	Phloroglucide from 50 cc. *A*, grms.
6 a.m.	49·78	67·42	117·20	0·0370	+0·279°	0·1068	−0·001°	0·1094	−0·001°	0·0043
8 a.m.	54·85	74·27	129·12	0·0647	+0·215	0·1659	−0·063	0·1662	−0·061	0·0065
10 a.m.	56·45	81·76	138·21	0·0978	+0·250	0·2172	−0·044	0·2168	−0·049	0·0078
12 noon	52·00	76·25	128·25	0·0895	+0·240	0·2000	−0·034	0·2049	−0·039	0·0062
2 p.m.	51·57	86·66	138·23	0·0882	+0·266	0·2044	−0·075	0·2147	−0·085	0·0070
4 p.m.	49·60	83·70	133·30	0·0584	+0·322	0·1726	±0·000	0·1756	±0·000	0·0068
6 p.m.	55·47	82·90	138·37	0·0538	+0·267	0·1594	−0·014	0·1704	−0·044	0·0082
8 p.m.	47·31	80·40	127·71	0·0444	+0·247	0·1243	−0·003	0·1338	−0·006	0·0048
10 p.m.	54·00	79·60	133·60	0·0412	+0·183	0·1200	−0·010	0·1250	−0·012	0·0056
12 night	43·72	71·48	115·20	0·0320	+0·186	0·0959	−0·011	0·1103	−0·011	0·0058
2 a.m.	44·19	70·25	114·44	0·0252	+0·157	0·0720	−0·008	0·0803	−0·002	0·0048
4 a.m.	39·70	64·72	104·42	0·0213	+0·107	0·0590	+0·005	0·0718	+0·003	0·0066

* This polarisation refers to the diluted solution after inversion, the dilution being twice that of *A*.

Mangold Stalks. August 26th–27th, 1913.

The extract was concentrated *in vacuo* and made up to 100 cc., 70 cc. of this being treated with basic lead acetate and washed to nearly 500 cc. The excess of lead was removed by solid Na_2CO_3 and solution made to 500 cc. = *A*.

Time		Dry matter sol. in alcohol (vacuum-dried), grms.	Dry matter insol. in alcohol (vacuum-dried), grms.	Total vacuum-dried matter (T.V.D.M.), grms.	Direct reducing power of 25 cc. *A*, grms. CuO	Polarisation of *A* in 200 mm. tube, α_D^{20}	Invertase inversion: Reduction of 25 cc. *A* after inversion, grms. CuO	Invertase inversion: α_D after inversion in 200 mm. tube	Citric inversion: Reduction of 25 cc. *A* after inversion, grms. CuO	Citric inversion: α_D after inversion in 200 mm. tube	Phloroglucide from
6 a.m.	Tops ...	10·17	9·06	19·23	0·1114	+0·302°	0·1836	+0·044°	0·1700	+0·084°	0·01
6 a.m.	Bottoms	15·45	13·00	28·45	0·2533	+0·556	0·3501	+0·153	0·3398	+0·153	0·00
Noon.	Tops ...	11·07	9·10	20·17	0·1893	+0·100	0·2660	+0·040	0·2706	+0·048	0·00
Noon.	Bottoms	16·22	11·64	27·86	0·3440	+0·557	0·4412	+0·136	0·4093	+0·177	0·02
6 p.m.	Tops ...	12·79	11·05	23·84	0·1880	+0·187	0·2714	+0·072	0·2760	+0·071	0·01
6 p.m.	Bottoms	19·86	14·51	34·37	0·2745	+0·654	0·3721	+0·085	0·3707	+0·090	0·02
12 night.	Tops ...	9·51	9·29	18·80	0·1260	+0·282	0·1936	+0·024	0·1960	+0·019	0·00
12 night.	Bottoms	14·68	11·63	26·31	0·2172	+0·448	0·3138	+0·122	0·2958	+0·144	0·00

Series II. Mangold Leaves. September 10th–11th, 1912.

(1) Extract evaporated *in vacuo* and made to 250 cc. 200 cc. of the 250 treated with basic lead acetate, filtered and washed to 1 litre. Excess of lead removed by solid sodium carbonate and made to 1000 cc. = *A*.

Time	Dry matter sol. in alcohol (vacuum-dried), grms.	Dry matter insol. in alcohol (vacuum-dried), grms.	Total vacuum-dried matter, grms.	Direct reducing power of 5 cc. *A*, grms. CuO	Polarisation of *A* in 200 mm. tube, α_D^{20}	Invertase inversion		Citric inversion		Phloroglucide from 50 cc. *A*, grms.
						Reduction of 5 cc. *A* after inversion, grms. CuO	α_D after inversion in 200 mm. tube	Reduction of 5 cc. *A* after inversion, grms. CuO	α_D after inversion in 200 mm. tube	
10 a.m.	127·92	105·75	233·67	0·1380	+0·754°	0·2444	−0·325°	0·2486	−0·183°	0·0265

(2) Extract evaporated *in vacuo* and made to 250 cc. 200 cc. of the 250 treated with basic lead acetate, filtered and washed to 1 litre = *A*. 375 cc. of *A* were deprived of lead by solid Na_2CO_3 and made up to 500 cc. = *B*. For reduction used 10 cc. *B* = 7·5 cc. *A*. (Solution after inversion = *C*.)

Time	Dry matter sol. in alcohol (vacuum-dried), grms.	Dry matter insol. in alcohol (vacuum-dried), grms.	Total vacuum-dried matter, grms.	Direct reducing power of 7·5 cc. *A*, grms. CuO	Polarisation of *B* in 200 mm. tube, α_D^{20}	Invertase inversion		Citric inversion		Phloroglucide from 50 cc. *B*, grms.
						Reduction of 7·5 cc. *A* after inversion, grms. CuO	α_D after inversion in *C* (200 mm.)	Reduction of 7·5 cc. *A* after inversion, grms. CuO	α_D after inversion in *C* (200 mm.)	
1 p.m.	62·50	78·92	141·42	0·1655	+0·381°	0·2635	−0·228°	0·2730	−0·270°	0·0110

(3) Extract evaporated *in vacuo* and made up to 500 cc. 440 cc.[1] of the 500 treated with basic lead acetate, filtered and washed to nearly

[1] In some cases, indicated in Table under "Remarks," 460 cc. were used instead of 440 cc.

2 litres. Solid sodium carbonate added and solution made up to 2000 cc. = *A*.

Time	Dry matter sol. in alcohol (vacuum-dried), grms.	Dry matter insol. in alcohol (vacuum-dried), grms.	Total vacuum-dried matter, grms.	Volume of solution *A* used for reduction (*x*)	Polarisation of *A* in 200 mm. tube a_D^{20}	Reduction of *x* cc. of *A*, grms. CuO	Invertase inversion		Citric inversion		Phlorogluicide from 50 cc. *A*	Remarks
							Reduction of *x* cc. *A* after inversion, grms. CuO	a_D after inversion (200 mm.)	Reduction of *x* cc. *A* after inversion	a_D after inversion (200 mm.)		
4 p.m.	68·20	72·36	140·56	20 cc.	+0·215°	0·2434	0·3820	−0·107°	0·3840	−0·118°	0·0163	460 cc.
11 p.m.	67·60	68·08	135·68	20 cc.	+0·390	0·2294	0·4203	−0·160	0·4172	−0·165	0·0157	440 cc.
2 a.m.	66·88	65·10	131·98	15 cc.	+0·470	0·1862	0·3643	−0·235	0·3650	−0·195	0·0165	440 cc.
4 a.m.	80·50	76·40	156·90	15 cc.	+0·350	0·1926	0·3373	−0·200	0·3400	−0·220	0·0159	440 cc.
6 a.m.	62·10	68·66	130·76	25 cc.	+0·170	0·2445	0·3945	−0·154	0·3940	−0·143	0·0129	440 cc.
8 a.m.	61·51	73·15	134·66	20 cc.	+0·235	0·1753	0·3075	−0·147	0·3185	−0·144	0·0126	440 cc.

(4) Extract evaporated *in vacuo* and made up to 500 cc. 440 cc. (or 460 cc.) of the 500 treated with basic lead, filtered and washed to 2 litres = *A*. 30 cc. of *A* were precipitated with solid sodium carbonate to remove the lead and made up to 500 cc. = *B*. 25 cc. of *B* used for direct reduction.

Time	Dry matter sol. in alcohol, grms.	Dry matter insol. in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution *B* used for reduction (*x*)	Polarisation of *B* in 200 mm. tube $a_D^{20°}$	Reduction of *x* cc. of *B*, grms. CuO	Invertase inversion		Citric inversion		Phlorogluicide from 50 cc. *A*	Remarks
							Reduction of *x* cc. *B* after inversion, grms. CuO	a_D after inversion (200 mm.)	Reduction of *x* cc. *B* after inversion	a_D after inversion (200 mm.)		
6 p.m.	75·38	72·52	147·90	25 cc.	+0·135°	0·2333	0·3920	−0·162	0·3955	−0·228°	0·0098	460
8 p.m.	64·34	71·89	136·23	25 cc.	+0·130	0·1662	0·2863	−0·165	0·2938	−0·124	0·0161	440

Mangold Stalks. September 10th–11th, 1912.

Extract evaporated *in vacuo* and made up to 200 cc. 170 cc. (or 180 cc.) of the 200 treated with basic lead acetate, filtered and washed

to nearly 2 litres. Solid sodium carbonate added to precipitate the lead exactly and solution made to 2000 cc. = *A*.

Time	Dry matter sol. in alcohol, grms.	Dry matter insol. in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution *A* used for reduction (*x*)	Polarisation of *A* in 200 mm. tube $a_D^{20°}$	Reduction of *x* cc. of solution *A*, grms. CuO	Invertase inversion: Reduction of *x* cc. *A* after inversion, grms. CuO	Invertase inversion: a_D after inversion (200 mm.)	Citric inversion: Reduction of *x* cc. *A* after inversion, grms. CuO	Citric inversion: a_D after inversion (200 mm.)	Phlorogluciide from 50 cc. *A*	Remarks
10 a.m.	67·20	35·57	102·77	10 cc.	+0·636°	0·2279	0·2767	+0·228°	0·2862	+0·097°	0·0186	170 cc.
4 p.m.	43·86	24·69	68·55	20 cc.	+0·380	0·3828	0·4505	+0·021	0·4639	+0·069	0·0117	180 cc.
11 p.m.	38·44	20·32	58·76	20 cc.	+0·471	0·2836	—	—	0·3481	+0·055	0·0079	170 cc.
4 a.m.	52·64	29·29	81·93	15 cc.	+0·595	0·3288	0·3982	+0·090	0·4012	+0·124	0·0134	180 cc.
6 a.m.*	43·30	23·96	67·26	25 cc. *B*	+0·277	0·3045	0·3595	+0·062	0·3637	+0·048	0·0113	180 cc.

* In the 6 a.m. analysis, 300 cc. of *A* were treated with solid sodium carbonate and made up to 500 cc. (= *B*). The polarimetric data therefore refer to solution *B*.

Mangold Mid-ribs. September 10th–11th, 1912.

Extract evaporated *in vacuo* and made up to 100 cc. 80 cc. of the 100 cc. treated with basic lead acetate, filtered and washed to 500 cc. = Solution *A*. 150 cc. of Solution *A* deprived of lead by solid sodium carbonate and made up to 200 cc. = Solution *B*.

Time	Vacuum-dried matter sol. in alcohol, grms.	Vacuum-dried matter insol. in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution *B* used for reduction (*x*)	Polarisation of *B* in 200 mm. tube $a_D^{20°}$	Reduction of *x* cc. of solution *B*, grms. CuO	Invertase inversion: Reduction of *x* cc. *B* after inversion, grms. CuO	Invertase inversion: a_D after inversion (200 mm.)	Citric inversion: Reduction of *x* cc. *B* after inversion, grms. CuO	Citric inversion: a_D after inversion (200 mm.)	Phlorogluciide from 50 cc. *A*
0 a.m.	17·65	11·35	29·00	15 cc.	+0·636°	0·3025	0·3825	−0·028°	0·3858	−0·041°	0·0061
4 p.m.	12·43	7·36	19·79	20 cc.	+0·520	0·2715	0·3380	—	0·3489	+0·076	0·0101
1 p.m.	10·15	7·27	17·42	25 cc.	+0·382	0·2766	0·3618	—	0·3646	+0·006	0·0102
4 a.m.	15·05	8·16	23·21	25 cc.	+0·425	0·3385	0·4631	+0·042	0·4625	+0·059	0·0177
6 a.m.	12·90	8·50	21·40	25 cc.	+0·367	0·3446	0·4437	—	0·4455	+0·007	0·0130

Series III. Mangold Leaves. October 11th–12th, 1912.

Extract evaporated *in vacuo* and made up to 500 cc. 440 cc. of the 500 were treated with basic lead acetate, and the precipitate washed

until the volume of filtrate was exactly 2 litres = *A*. 300 cc. (or 250 cc.)* of the solution were precipitated by solid sodium carbonate and made up to 500 cc. = Solution *B*.

Time	Vacuum-dried matter sol. in alcohol, grms.	Vacuum-dried matter insol in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution *B* used for reduction (*x*)	Polarisation of *B* in 200 mm. tube $a_D^{20°}$	Reduction of *x* cc. of solution *B*, grms. CuO	Invertase inversion: Reduction of *x* cc. after inversion, grms. CuO	Invertase inversion: a_D after inversion (200 mm.)	Citric inversion: Reduction of *x* cc. after inversion, grms. CuO	Citric inversion: a_D after inversion (200 mm.)	Phloroglucide from 50 cc. *A*	Remarks
9 a.m.	65·97	60·42	126·39	25 cc. *B*	+0·212°	0·2289	0·3410	−0·084°	0·3469	−0·097°	0·0172	300 c
11 a.m.	81·80	67·88	149·68	25 cc. *B*	+0·217	0·2524	0·3943	−0·132	0·3997	−0·119	0·0242	250 c
1 p.m.	75·09	67·72	142·72	25 cc. *B*	+0·113	0·2503	0·3825	−0·152	0·3892	−0·141	0·0233	250 c
3 p.m.†	97·94	80·34	178·28	10 cc. *A*	+0·690	0·2145	0·3906	−0·344	0·3870	−0·273	0·0281	*A* dir
5 p.m.	76·95	69·48	146·43	25 cc. *B*	+0·189	0·2427	0·3897	−0·141	0·3941	−0·165	0·0221	250 c
7 p.m.	92·74	79·09	171·83	25 cc. *B*	+0·304	0·2286	0·4058	−0·131	0·4048	−0·125	0·0288	200 c
9 p.m.	73·53	62·23	135·76	25 cc. *B*	+0·194	0·2345	0·3670	−0·098	0·3715	−0·100	0·0194	250 c
11 p.m.	64·63	70·16	134·79	25 cc. *B*	+0·274	0·2210	0·3706	−0·071	0·3710	−0·064	0·0149	300 c
1 a.m.	73·71	66·34	140·05	25 cc. *B*	+0·230	0·2614	0·4120	−0·157	0·4225	−0·153	0·0192	300 c
3 a.m.	76·25	60·25	136·50	25 cc. *B*	+0·263	0·2868	0·4688	−0·168	0·4683	−0·171	0·0156	300 c
5 a.m.	74·72	61·92	136·64	25 cc. *B*	+0·279	0·2679	0·4190	−0·121	0·4202	−0·125	0·0155	300 c
7 a.m.†	66·36	62·36	128·72	20 cc. *A*	+0·234	0·2818	0·4165	−0·198	0·4220	−0·188	0·0119	*A* dir

* Whether 250 cc. or 300 cc. of the 500 cc. were used is shown in the last column. At 7 p.m. 200 cc. of *A* were taken.

† In these analyses the solution *A* was used direct, without further dilution, so that the polarimetric and reduction data refer to the solution *A*.

Mangold Stalks. October 11th–12th, 1912.

Extract evaporated *in vacuo* and made up to 200 cc. 170 cc. of the 200 precipitated by basic lead acetate, filtered and washed to 2 litres = Solution *A*. 300 cc. of *A* treated with solid sodium carbonate and made up to 500 cc. = *B*.

Time	Vacuum-dried matter sol. in alcohol, grms.	Vacuum-dried matter insol. in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution *B* used for reduction (*x*)	Polarisation of *B* in 200 mm. tube $a_D^{20°}$	Reduction of *x* cc. of solution *B*, grms. CuO	Invertase inversion: Reduction of *x* cc. after inversion, grms. CuO	Invertase inversion: a_D after inversion (200 mm.)	Citric inversion: Reduction of *x* cc. after inversion, grms. CuO	Citric inversion: a_D after inversion (200 mm.)	Phloroglucide from 50 cc. *A*	Remarks
11 a.m.	43·70	25·67	69·37	25 cc. *B*	+0·332°	0·2865	0·3417	+0·045°	0·3483	+0·040°	0·0095	300 cc. *A*
11 p.m.*	40·16	25·56	65·72	25 cc. *A*	+0·492	0·3735	0·4605	+0·102	0·4663	+0·093	0·0110	*A* used dir

* Solution *A* used direct without further dilution, so that polarimetric and reduction data refer to solution *A*.

Mangold Mid-ribs. October 11th–12th, 1912.

Extract evaporated *in vacuo* and made up to 100 cc. 70 cc. of the 100 cc. precipitated by basic lead acetate, filtered and washed to 1 litre = Solution *A*. 300 cc. of *A* precipitated by solid Na_2CO_3 and made up to 500 cc. = *B*.

Time	Vacuum-dried matter sol. in alcohol, grms.	Vacuum-dried matter insol. in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution *B* used for reduction (*x*)	Polarisation of *B* in 200 mm. tube $a_D^{20°}$	Reduction of *x* cc. *B*, grms. CuO	Citric inversion: Reduction of *x* cc. after inversion, grms. CuO	Citric inversion: a_D after inversion (200 mm.)	Phloroglucide from 50 cc. *A*	Remarks
11 a.m.	10·56	5·95	16·51	25 cc. *B*	+0·100°	0·1032	0·1343	−0·007°	0·0056	300 cc. *A* to 500
11 p.m.*	9·07	6·15	15·26	25 cc. *A*	+0·214	0·1503	0·2032	+0·006	0·0039	*A* used direct

* In the 11 p.m. analysis 80 cc. (instead of 70 cc.) of original 100 cc. were taken; the solution *A* was treated with solid sodium carbonate and used direct without further dilution.

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STUDIES OF THE FORMATION AND TRANSLOCATION OF CARBOHYDRATES IN PLANTS.

II. THE DEXTROSE-LAEVULOSE RATIO IN THE MANGOLD

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Brown and Morris [1893] in their well-known experiments on the *Tropæolum* leaf observed that the hexoses of the leaf instead of being present in the proportion corresponding with invert sugar, invariably appeared to consist very largely of laevulose. In several cases dextrose was entirely absent, whilst in others the proportion of laevulose to dextrose varied from about 6 : 1 down to about 2 : 1. As they had concluded on other grounds that the reducing sugars are formed by inversion from cane sugar, they explained the predominance of laevulose as being due to the dextrose being "more readily put under contribution for the respiratory processes of the cell than is laevulose."

Lindet (1900) made a special study of the proportion of the hexoses present in the leaf and leaf-stalks of the sugar beet at different periods of growth. His analyses showed that in normally growing leaves, especially in the earlier stages of growth (July 3rd to 24th), the proportion of dextrose was generally slightly *greater* than that of laevulose, although on several occasions it was slightly *less*; thus on July 3rd and July 24th, for example, it was found that the ratio of laevulose to dextrose was 1·3 and 1·11 respectively. On the other hand and in striking contrast to the leaves, the laevulose in the leaf-stalks was *invariably* found to form only a small proportion of the dextrose present, varying from 0 to 35 per cent. Lindet adopts Brown and Morris' views to explain these results and concludes that the excess of laevulose in the leaves is due to the dextrose being consumed in these tissues by *respiration* more rapidly than the laevulose; on the other hand the laevulose has been

removed from the sap of the stalks in forming new tissue, laevulose being the sugar specially adapted to this purpose. Lindet in a recent paper [1911], inspired by the results of his earlier work, cites experiments made with yeasts which also serve to show that laevulose lends itself to reproductive growth or new tissue formation better than dextrose.

Parkin [1912] in experiments on the snowdrop leaf found that in 47 out of 52 analyses the laevulose was in excess of the dextrose; representing laevulose as unity, in these cases the ratio varied from 1 : 0·4 to 1 : 0·76. In the five remaining cases, the ratio was 1 : 1·01 to 1 : 1·06. The ratio of laevulose to dextrose appeared to rise during the night, that is when photosynthesis is in abeyance; the excess of laevulose was always greatest in the lower (colourless) part of the long snowdrop leaf. To explain his results Parkin also adopted Brown and Morris' view that the dextrose lends itself most readily to the respiratory needs of the plant, whilst the laevulose is used largely in constructive work such as the building up of the plant's framework.

As was pointed out by Brown and Morris in 1893 the correctness of the dextrose and laevulose values depends entirely on the accuracy of the readings of the rotatory power; a slight error in these makes a large difference in the apparent proportion of the two hexoses. The main purpose of the present paper is to show that, whilst it is possible to take the actual readings very accurately (in our case the probable error did not exceed 0·005°), the values are falsified, in the case of most plant material, by the presence of optically active substances other than the sugars, which are not completely precipitated by the basic lead acetate (or other defecating substance) used to purify the solutions. Typical substances of this kind are amino-acids and amides, such as glutamic acid and glutamine, aspartic acid and asparagine. The first three of these have a pronounced positive rotation which is greatly enhanced by acids, whilst asparagine is laevo-rotatory in aqueous solution and dextro-rotatory in acid solution. The influence of these substances in falsifying the results obtained by the method of double polarisation for the cane sugar in the molasses of sugar manufacture has been studied by several chemists, more particularly by H. Pellet (compare *Dosage du Sucre par Inversion*, 1913), but the effect of these and other impurities on the results obtained for the dextrose : laevulose ratio in plant material has not hitherto been taken into account.

We find in the *mangold*, just as Lindet did in the sugar beet, that the dextrose always seems to be in excess of the laevulose, especially in the

mid-ribs and stalks (where the ratio $\frac{D}{L}$ is often extremely high), whereas in the *potato* the reverse holds, the laevulose apparently predominating as in the cases studied by Brown and Morris, and by Parkin. At the same time it can be shown that the apparent excess of dextrose or laevulose is correlated with certain abnormalities in the cane sugar estimations, caused by the presence of optically active impurities. The apparent excess of *dextrose* in the tissues of certain plants (sugar beet and mangold) is indeed due to the presence of a dextro-rotatory impurity (possibly glutamine), whilst the predominance of laevulose in other plants (e.g. tropæolum, snowdrop, potato) is to be attributed to a laevo-rotatory impurity (e.g. asparagine).

In the mangold the difference between the results obtained for saccharose by the reduction method and by the double polarisation method, which we have referred to in the preceding paper (p. 273), is always far greater in the stalks and mid-ribs than in the leaves, a fact which we attribute to the accumulation of optically active impurities in these parts. Side by side with this we have the fact that, whilst in the leaves the ratio of dextrose to laevulose $\left(\frac{D}{L}\right)$ is in general not very far removed from unity, in the stalks and mid-ribs the ratio $\frac{D}{L}$ is very much greater, generally varying from 2·5 to 10. This ratio, too, is far higher in the bottom halves of the stalks than in the top halves, pointing to an accumulation in the lower part of the stalks of the dextro-rotatory impurity. Striking differences are also found between the results for cane sugar in the top and bottom halves, according as they are calculated from the reduction data or from the polarimetric values. Thus in the *top* halves the results obtained by polarisation may be 80 to 90 per cent. *lower* than the values obtained by reduction, whilst in the *bottom* halves they are *high* by 40 per cent. As the day proceeds, the relation of tops and bottoms may be reversed, the impurity which was in the top half passing down to the lower part of the stalks (compare the values at noon, 6 p.m. and midnight given on p. 344, Table VIII).

Independently of any error which may be caused by the improper use of basic lead acetate (see p. 270), a difficulty which makes it impossible to obtain really accurate values of the proportion of dextrose and laevulose lies in the fact that allowance has to be made in the calculation for the reducing power and rotation of the pentoses which are invariably present in the alcoholic extracts prepared by our method of working.

In any particular case, one is entirely ignorant as to the nature of these; if it be assumed that they consist of arabinose and xylose, whilst it is possible to introduce a fairly accurate correction for the reducing power, owing to the fact that the reducing power of arabinose is nearly identical with that of xylose (Daish [1914]), this is not the case for the rotatory power as $[\alpha]_D$ has widely different values for the two pentoses (for arabinose $[\alpha]_D^{20^\circ} = +122^\circ$, for xylose $[\alpha]_D^{20^\circ} = +18{\cdot}78^\circ$). This large disparity in the specific rotatory powers may, in certain cases, involve a difference of $0{\cdot}1^\circ$ or more in the rotation from which the dextrose and laevulose are calculated, according as the pentose is assumed to be arabinose or xylose respectively; in the example given, showing our method of calculation (see p. 317), the difference is only $0{\cdot}041^\circ$, but it is frequently much greater and then represents quite a large proportion of the actual angle used in calculating the reducing sugars. On this account, in default of information as to the exact nature of the pentoses present, we have always calculated the dextrose and laevulose on the two assumptions: (1) that the pentose is arabinose, (2) that the pentose is xylose. But it is quite possible that the pentose really present may consist to a greater or less extent of one of the less known pentoses, e.g. *d*-ribose, and if this is the case the results for dextrose and laevulose will be correspondingly at fault.

Dextrose and laevulose are, moreover, calculated from values obtained after allowing for all the other substances present—cane sugar, pentoses, maltose (if present). The degree of accuracy obtained will naturally depend on the accuracy with which the other constituents have been estimated. Even the difference caused by calculating the small proportion of pentose as arabinose or as xylose may, as for example in the mangold leaf, 5 p.m., October 11th, make the ratio $\frac{D}{L}$, which appears to be strictly 1·00 when the pentose is taken as xylose, have a very different value (0·844) when the pentose is assumed to be arabinose.

In putting forward the results given in this paper, I am conscious that the values given as dextrose and laevulose probably do not, in most cases, represent real values; they are therefore designated "apparent dextrose" and "apparent laevulose." Although little confidence can be placed in them as *absolute* values for these sugars, they show a regular variation which is sufficiently striking to justify detailed consideration. This variation may be due either to a real variation of the dextrose and laevulose or, what is more probable, to a regular

variation in the amount of the optically active impurities which are present; if the latter, the fluctuation of these substances during the 24 hours must be quite as great as the fluctuation of the sugars themselves. Until a method has been devised by which it is possible to estimate accurately the true proportions of dextrose and laevulose, when present together, without having recourse to polarimetric data, the facts brought forward in this paper, and that which follows, show that it is impossible to know with any certainty the real proportions of these sugars present in different plant tissues; it is, therefore, equally impossible to draw conclusions as to the function of these two sugars in the plant—whether the one is more suited than the other to build up new tissue or whether one is more easily put under contribution than the other in respiration. The fermentation test, which Parkin and others have used to ascertain whether the solutions they analysed were free from optically active substances other than sugars, is one which is by no means reliable for this purpose. Parkin, whose work in most other respects is valuable, considered that, as the solutions prepared from the snowdrop leaves showed, after fermentation with yeast, a negligible rotatory and reducing power, *no other substances likely to possess these properties were present in the original solutions.* It would be quite possible for large amounts of asparagine to have been present, sufficiently large indeed to explain the apparent preponderance of laevulose in the snowdrop (where the ratio of laevulose to dextrose varied from 1 : 0·4 to 1 : 0·76) and yet to have escaped detection by this method, as the asparagine would be largely, if not entirely, consumed by the yeast in its growth; asparagine indeed is used very largely as a nutrient material for yeasts, for example in Hayduck's solution.

EXPERIMENTAL.

The methods of analysis have been described in the preceding paper; an example is there given of the method of calculating the proportions of dextrose and laevulose according as the pentoses are assumed to be arabinose or xylose (see p. 318). The actual data used are given on pp. 319–325; the results are calculated on the *total vacuum-dried matter* of the leaf.

In the tables which follow, D = per cent. of "apparent dextrose" in the total vacuum-dried matter; L = per cent. of "apparent laevulose" in the total vacuum-dried matter.

TABLE I.

Mangold Leaves. Series I. August 26th–27th, 1913.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose taken as		Hexoses calculated as invert sugar %	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
6 a.m. ...	0·73	Nil	∞	0·73	Nil	∞	0·73	0·73	0·77	
8 a.m. ...	0·64	0·77	0·83	0·87	0·52	1·68	1·41	1·39	1·42	
10 a.m. ...	1·09	1·06	1·02	1·32	0·80	1·65	2·15	2·12	2·16	
12 noon ...	1·11	1·02	1·08	1·33	0·78	1·70	2·13	2·11	2·15	Day
2 p.m. ...	1·01	0·90	1·12	1·24	0·66	1·88	1·91	1·90	1·94	
4 p.m. ...	0·92	0·21	4·34	1·15	0·00	∞	1·13	1·15	1·18	
6 p.m. ...	0·49	0·46	1·07	0·74	0·19	3·87	0·95	0·93	0·96	Sun sets 7 p.m
8 p.m. ...	0·73	0·14	5·33	0·92	0·00	∞	0·87	0·92	0·90	
10 p.m. ...	0·29	0·45	0·65	0·50	0·23	2·17	0·74	0·73	0·74	
12 night ...	0·32	0·24	1·35	0·55	0·00	∞	0·56	0·55	0·57	Night
2 a.m. ...	0·29	0·08	3·62	0·36	0·00	∞	0·37	0·36	0·38	
4 a.m. ...	0·00	0·20	0·00	0·16	0·02	7·14	0·20	0·19	0·20	Sun rises 5.5 a.m.

TABLE II.

Mangold Leaves. Series II. September 10th–11th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
10 a.m. ...	2·72	3·01	0·904	2·91	2·80	1·039	5·73	5·71	5·72	
1 p.m. ...	3·85	3·62	1·063	4·06	3·37	1·204	7·47	7·43	7·50	Day
4 p.m. ...	3·11	3·87	0·804	3·47	3·46	1·003	6·98	6·93	7·00	
6 p.m. ...	3·59	5·38	0·668	3·83	5·11	0·749	8·97	8·94	8·90	Sun sets 6.26 p.m.
8 p.m. ...	2·56	4·25	0·602	2·95	3·74	0·789	6·81	6·69	6·76	
11 p.m. ...	3·34	3·79	0·881	3·73	3·36	1·110	7·13	7·09	7·10	Night
2 a.m. ...	3·43	4·39	0·781	3·84	3·93	0·977	7·82	7·77	7·81	
4 a.m. ...	3·17	3·73	0·851	3·51	3·36	1·045	6·90	6·87	6·91	Sun rises 5.30 a.m.
6 a.m. ...	2·64	3·68	0·717	2·99	3·30	0·906	6·32	6·29	6·30	Day
8 a.m. ...	2·26	3·15	0·718	2·60	2·78	0·935	5·41	5·38	5·38	

TABLE III.

Mangold Leaves. Series III. October 11th–12th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
9 a.m.	5·62	4·67	1·203	6·07	4·18	1·450	10·29	10·25	10·32	
11 a.m.	5·32	6·35	0·839	5·82	5·81	1·001	11·67	11·63	11·62	
1 p.m.	5·17	7·03	0·735	5·67	6·49	0·874	12·20	12·16	12·12	Day
3 p.m.	4·79	5·43	0·882	5·26	4·91	1·070	10·22	10·17	10·24	
5 p.m.	5·24	6·21	0·844	5·72	5·69	1·005	11·45	11·41	11·46	Sun sets 5.15 p.m
7 p.m.	5·67	5·82	0·975	6·17	5·27	1·171	11·49	11·44	11·47	
9 p.m.	5·92	6·04	0·980	6·38	5·54	1·151	11·96	11·92	11·98	
11 p.m.	5·04	4·32	1·165	5·41	3·91	1·385	9·36	9·32	9·39	
1 a.m	5·25	5·53	0·950	5·69	5·04	1·129	10·78	10·73	10·78	Night
3 a.m.	6·05	6·37	0·950	6·44	5·95	1·084	12·42	12·39	12·41	
5 a.m.	6·26	5·15	1·215	6·64	4·73	1·404	11·41	11·37	11·49	Sun rises 6.21 a.m.
7 a.m.	4·81	4·80	1·002	5·15	4·44	1·160	9·61	9·59	9·62	Day

TABLE IV.

Mangold Leaf-stalks. Series I. Top and Bottom Halves.
August 26th–27th, 1913.

Time		Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %	
		D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
6 a.m.	Tops ...	4·36	0·76	5·72	5·09	0·00	∞	5·12	5·09	5·35	
	Bottoms	7·90	0·94	8·37	8·55	0·23	37·2	8·84	8·78	9·11	
12 noon	Tops ...	4·89	5·04	0·97	5·37	4·51	1·19	9·93	9·88	9·97	Day
	Bottoms	10·20	2·70	3·77	11·00	1·83	6·01	12·90	12·83	13·17	
6 p.m.	Tops ...	4·22	3·61	1·17	4·87	2·89	1·68	7·83	7·76	7·89	
	Bottoms	8·45	1·71	4·95	9·10	1·00	9·10	10·16	10·20	10·47	Sun sets 7 p.m.
12 night	Tops ...	5·09	1·29	3·95	5·71	0·61	9·35	6·38	6·32	6·61	Night
	Bottoms	6·80	1·37	4·97	7·38	0·73	10·05	8·17	8·11	8·49	

TABLE V.

Mangold Leaf Mid-ribs. Series II. September 10th–11th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$%, pentose as		Hexoses calculated as invert sugar %	
	D%	L%	$\frac{D}{L}$	D%	L%	$\frac{D}{L}$	Arabinose	Xylose		
10 a.m. ...	17·8	5·3	3·36	18·0	5·1	3·53	23·1	23·1	23·6	Day
4 p.m. ...	17·9	4·1	4·37	18·4	3·5	5·26	22·0	21·9	22·6	
11 p.m. ...	15·3	4·9	3·12	15·8	4·3	3·68	20·2	20·1	20·6	Night
4 a.m. ...	12·9	5·8	2·22	13·6	5·1	2·67	18·7	18·7	19·0	
6 a.m. ...	14·4	6·7	2·15	15·2	5·9	2·58	21·1	21·1	21·4	Day

TABLE VI.

Mangold Stalks. Series II. September 10th–11th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$%, pentose as		Hexoses calculated as invert sugar %	
	D%	L%	$\frac{D}{L}$	D%	L%	$\frac{D}{L}$	Arabinose	Xylose		
10 a.m. ...	14·8	5·1	2·90	15·3	4·6	3·33	19·9	19·9	20·5	Day
4 p.m. ...	17·5	8·45	2·07	18·1	7·75	2·34	25·9	25·9	26·3	
11 p.m. ...	17·3	4·5	3·84	17·8	3·9	4·56	21·8	21·7	22·4	Night
4 a.m. ...	17·3	6·0	2·88	17·8	5·4	3·30	23·3	23·2	23·7	
6 a.m. ...	19·1	7·6	2·51	19·75	6·95	2·84	26·7	26·7	26·7	Day

TABLE VII.

Mangold Stalks and Mid-ribs. Series III. October 11th–12th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose	
Mid-ribs:									
11 a.m. ...	15·7	7·2	2·18	16·1	6·7	2·40	22·9	22·8	23·4
11 p.m. ...	14·0	4·3	3·11	14·5	3·9	3·73	18·5	18·4	19·0
Stalks:									
11 a.m. ...	19·3	5·8	3·32	19·9	5·2	3·81	25·1	25·1	25·7
11 p.m. ...	16·15	4·85	3·33	16·8	4·2	4·04	21·0	21·1	21·4

DISCUSSION OF RESULTS.

A. *Leaves.*

Series I. In Series I, Table I, the actual percentages of total hexoses are very small, especially at night, during which they range from 0·90 to 0·20 per cent.; consequently, even the small differences in the rotation of the pentoses, according as they are assumed to be arabinose or xylose, lead to considerable differences in the proportions of dextrose and laevulose apparently present. Thus, for example, at 8 a.m., if the pentose is assumed to be arabinose, laevulose appears to be in excess of the dextrose, the ratio $\frac{D}{L}$ being 0·83; if the pentose be taken as xylose, the dextrose appears in excess, the ratio $\frac{D}{L}$ becoming 1·68. In this particular case the rotation of the pentose represented, in a 200 mm. tube at 20°, as arabinose a reading of + 0·049°, as xylose + 0·009°, whilst, after allowing for the saccharose and pentoses present (see method of calculation, p. 317), the rotation left for the hexoses was − 0·042° or − 0·002° in the two cases.

That dextro-rotatory impurities are present in this series to an extent sufficient to invalidate the calculations and so to convey a false idea of the true quantities of dextrose and laevulose, is shown by considering the data obtained at 6 a.m. In this case, if the *whole* of the reducing sugar, obtained from the reduction data, is calculated as dextrose,

a rotation + 0·039° (in 200 mm. tube at 20°) is obtained, whereas the rotation actually observed, after allowing for the cane sugar and pentoses, was + 0·093° (pentoses as arabinose) or + 0·125° (pentoses as xylose). Thus in the two cases a *positive* rotation remains unaccounted for, of + 0·054° or + 0·086° respectively. Similarly at 4 p.m., the whole of the reducing sugars calculated as dextrose would barely account for the actual positive rotation observed if the pentoses be taken as xylose, the excess being + 0·004°; if, however, the pentoses be taken as arabinose, the dextrose would more than account for the rotation observed by + 0·037°, and in this case the dextrose becomes 0·92 per cent. and laevulose 0·21 per cent., the ratio $\frac{D}{L}$ being 4·34. Similarly at 8 p.m. the assumption that the whole of the reducing sugar is dextrose leaves + 0·008° unaccounted for if the pentose is xylose; when it is taken as arabinose, D becomes 0·73 and $L = 0·14$ per cent., the ratio $\frac{D}{L}$ being high, viz. 5·33. Similar observations hold for midnight, 2 a.m. and 4 a.m.; at 4 a.m. the quantity of reducing sugars is so small, that assuming the pentose to be arabinose causes the laevulose to appear in excess, whilst if it is taken as xylose, the dextrose appears largely in excess, $\frac{D}{L}$ being 7·14.

It is clear therefore that little significance can be attached to the values for dextrose and laevulose in Series I owing to the presence of a dextrorotatory impurity[1], the rotation of which is large relatively to that of the small quantity of sugars present. Even though this is the case, between 8 a.m. and 2 p.m. the values of dextrose and laevulose are approximately equal, especially when the pentoses are assumed to be arabinose, the ratio $\frac{D}{L}$ being approximately 1. It should be noted

[1] A determination was made for us by Mr E. Horton of the amino-nitrogen in the Solution *A* used in estimating the sugars in the case of a sample of mangold leaves picked at 2.45 p.m. on October 8th, 1914. 2 c.c. of this solution gave in the van Slyke micro-apparatus 0·225 c.c. of nitrogen at 0 and 760 representing 0·007 grm. of amino-nitrogen per 100 c.c. Calculated as *glutamine* this would represent 1·2 per cent. of glutamine on the total vacuum-dried weight of the leaf at a time of day when, judging by the results of Table III, the proportion of such impurity is at its minimum; in this picking, the cane sugar was 7·5 per cent. and the hexoses 19·1 per cent. of the total vacuum-dried matter, so that the proportion of the optically active amide is in this case only small relatively to the sugars, a fact which would explain that the ratio $\frac{D}{L}$ keeps in the neighbourhood of unity (see Tables II and III).

that at these times the proportion of the sugars is greatest, so that the effect of the rotation of the optically active impurity in falsifying the results is least marked. At night, apparently, an excess of this impurity is liberated, possibly as a waste product of metabolism, owing to degradation changes predominating, so that the laevulose appears to have disappeared entirely at 6 a.m., and a relatively large *positive* rotation remains unaccounted for even when the whole of the reducing sugar is assumed to be dextrose.

Fig. 1 shows the variation of "apparent dextrose" and "apparent laevulose" on the assumption that the pentoses are xylose[1] during the 24 hours, August 26th–27th. Throughout this period the dextrose

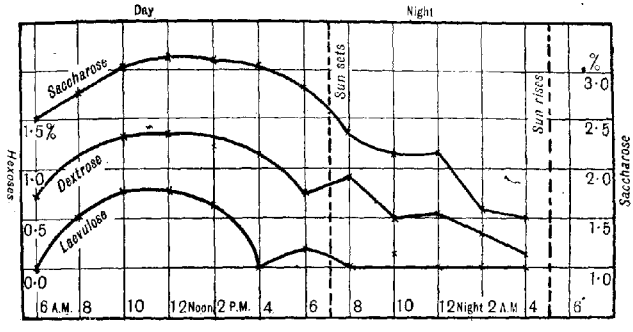

Fig. 1. Mangold leaves, apparent dextrose and laevulose, Series I, Aug. 26–27, 1913 (pentose as xylose).

curve is above the laevulose; during the period of actual insolation the curves are approximately parallel to each other and to the saccharose curve. If it were not for the presence of the dextro-rotatory impurity, the two curves would probably nearly coincide—the dextrose curve being lowered and the laevulose curve being correspondingly raised. On the assumption that the pentose is arabinose, the two curves actually coincide without any such correction being made. The parallelism of the curves of dextrose and laevulose with that of cane sugar is particularly striking when the apparent steepness of the curve of total hexoses is taken into account (see previous paper, Fig. 4), from which it might

[1] Throughout this paper the curves of dextrose and laevulose are drawn only for the case when the pentose is assumed to be xylose. The curves obtained by assuming the pentose to be arabinose are strictly parallel to these curves but slightly higher or lower; the effect of taking the pentose as arabinose instead of xylose is to raise the laevulose curve and lower the dextrose curve.

be inferred that the hexoses are formed more rapidly than the saccharose, and consequently precede it; instead, the quantity of each of the reducing sugars is roughly proportional to the cane sugar present, a fact which points to the formation of the hexoses from this sugar.

Series II. In Series II (Table II) the sugar percentages are far higher than in Series I and the fluctuations in the value $\frac{D}{L}$ are far less marked in consequence. With few exceptions (e.g. at 6 p.m. and 8 p.m.) the value of $\frac{D}{L}$ does not differ much from 1, the percentages of dextrose and laevulose being as nearly equal as one could expect bearing in mind the errors to which the calculations are subject. In general, the values of $\frac{D}{L}$ obtained by assuming the pentose to be arabinose are slightly *lower* than unity, whilst by assuming it to be xylose, they become slightly *higher* than unity. It is probable that $\frac{D}{L}$ would be almost exactly 1 were it not for the presence of small quantities of optically active impurities, which in some cases increase the value, in others lower it. The following table shows that the departure of the value $\frac{D}{L}$ from 1 goes hand in hand with the divergence Δ between the values for cane sugar found by the reduction and double polarisation methods; this divergence is no doubt also caused by the presence of these optically active impurities (see p. 344).

$\frac{D}{L}$ approximately 1			$\frac{D}{L}$ divergent from 1		
Time	$\frac{D}{L}$	Δ*	Time	$\frac{D}{L}$	Δ
10 a.m. ...	1·039	−14·0 %	1 p.m. ...	1·204	+25·9 %
4 p.m. ...	1·003	+ 1·5	6 p.m. ...	0·749	+15·2
11 p.m. ...	1·110	+ 7·2	8 p.m. ...	0·789	+23·2
2 a.m. ...	0·977	+ 7·4			
4 a.m. ...	1·045	+16·1			
6 a.m. ...	0·906	+10·0			
8 a.m. ...	0·935	+10·6			

* Δ represents the difference between the *reduction* and *polarisation* values for *cane sugar*, expressed as a percentage of the *average* value found by reduction by the invertase and citric acid methods; thus, e.g., +10·0 per cent. shows that the average value found by the double polarisation method is 10 per cent. higher than the average value found by reduction. In this particular case (6 a.m.) the cane sugar found by reduction was 4·24 per cent. on T.V.D.M., and by double polarisation 4·65 per cent.

The closer the value $\frac{D}{L}$ approximates to 1, the closer is the agreement between the cane sugar values by the two methods; at 4 p.m. particularly, when the value $\frac{D}{L}$ is practically 1, the divergence Δ is exceedingly small, viz. 1·5 per cent. only. It would appear that at this time the amount of optically active impurities influencing the results is practically *nil*. On the other hand, at 1 p.m., 6 p.m., and 8 p.m., when the value of $\frac{D}{L}$ departs considerably from unity, *in either direction*, there is a correspondingly large difference in the results for cane sugar by the two methods, the polarisation results being from 15 to 25 per cent. high. The figures at 6 p.m. and 8 p.m. are particularly interesting because at these times there is apparently an excess of laevulose[1], pointing to the presence of a *laevo-rotatory*, not a dextro-rotatory, impurity such as was present in the earlier part of the day (e.g. at 1 p.m.). At the same time, however, the polarisation figures are still higher than the reduction figures, showing that the *change* of rotation which accompanies the inversion process involves probably transformation of the laevo-rotatory substance into a compound with a still greater laevo-rotation—such as would happen, for example, in the transformation of asparagine into aspartic acid.

It is interesting to consider the curves in Fig. 2 showing the variation of the "apparent dextrose" and "apparent laevulose" during the 24 hours. Although these curves probably do not show the variation of the true sugars so much as that of the optically active impurities, they exhibit a regularity which points to the latter substances being formed regularly and progressively. In general the apparent dextrose and apparent laevulose increase when the cane sugar increases and fall when this sugar falls. The dextrose curve rises and falls three times in succession during the 24 hours, the rise and fall in the night (8 p.m. to 8 a.m.) being far more gradual and regular than the two abrupt changes during the day. The laevulose curve is less regular, rising gradually till 4 p.m., when D and L are practically equal; from this point the laevulose rises suddenly and follows the abrupt rise in the saccharose curve, which takes place just before sunset. The latter rise is therefore

[1] This apparent excess of laevulose would formerly have been explained by assuming that at these times the respiratory changes (utilising dextrose) predominate over the tissue-building changes. The polarisation data clearly point to abnormal quantities of optically active impurities at these points, which falsify the real values of D and L.

associated with a sudden increase in the amount of a *laevo-rotatory* impurity; at 4 p.m. the laevulose curve rises above the dextrose curve and remains above it until 10 p.m. From 6 p.m. to 8 p.m. $\frac{D}{L}$ has abnormally low values (0·749–0·789), but at 10 p.m. it is again practically unity, and it so remains throughout the night with very little change, although the laevulose curve rises and falls between 11 p.m. and 4 a.m., and falls between 4 a.m. and 8 a.m.

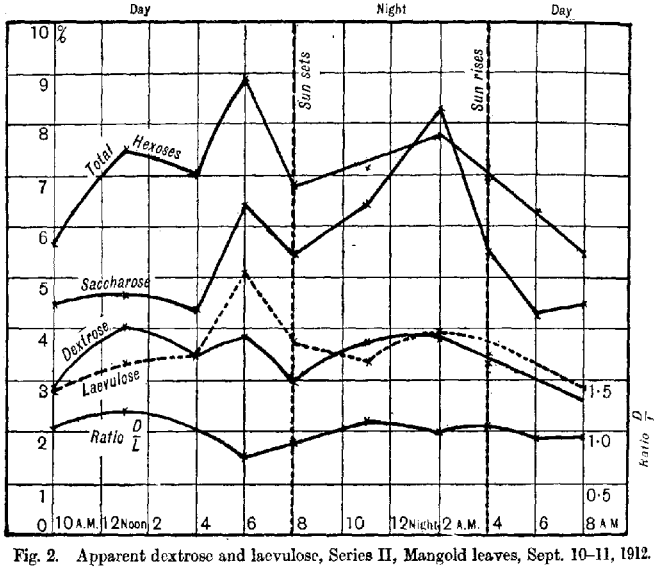

Fig. 2. Apparent dextrose and laevulose, Series II, Mangold leaves, Sept. 10–11, 1912.

The curve showing the variation of the ratio $\frac{D}{L}$ is also given in Fig. 2; it illustrates the marked periodic character of the fluctuations. These occur in two well-defined periods: In the first, a regular rise and fall of the ratio occurs in the eight hours from 10 a.m. to 6 p.m., during which the dextrose appears in excess; in the second period, from 6 p.m. to 10 p.m., the laevulose is in excess, but the ratio $\frac{D}{L}$ increasing. In the remaining 12 hours, from 10 p.m. to 10 a.m. there is little change in the value $\frac{D}{L}$ which remains very nearly unity.

Series III (Table III, Fig. 3). As in Series II, the ratio $\frac{D}{L}$ is, with few exceptions (9 a.m., 11 p.m. and 5 a.m.), approximately unity when the pentoses are taken as xylose; in most cases the ratio is greater than

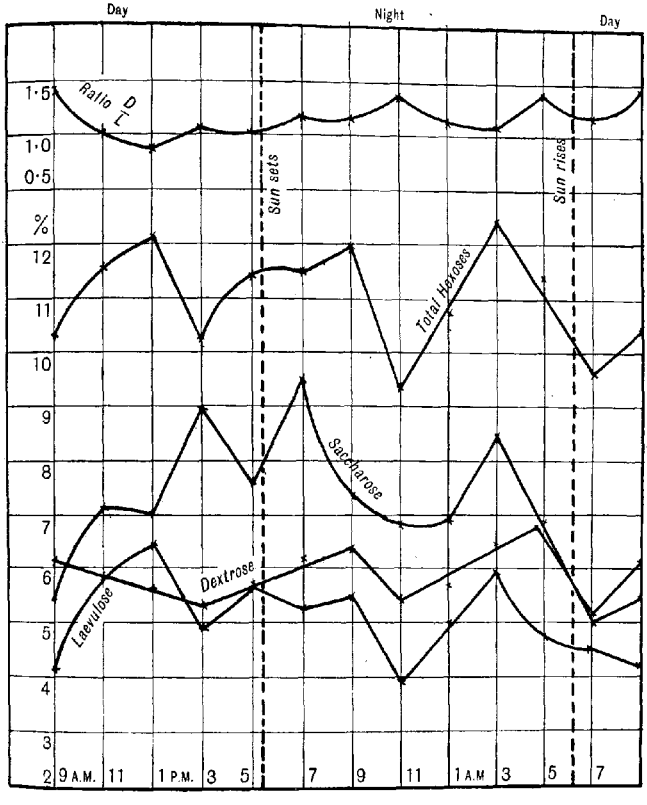

Fig. 3. Apparent dextrose and laevulose, Mangold leaves, Series III, Oct. 11–12, 1912.

1 when the pentoses are assumed to be xylose and less than 1 when they are taken as arabinose. As in Series II it can be shown that when the ratio $\frac{D}{L}$ most departs from unity (5 a.m., 7 a.m., 9 a.m.) the difference

Δ between the values found for saccharose by reduction and polarisation is greatest, the polarisation figures being 20 to 30 per cent. high.

During the greater part of the day (except at 9 a.m., when $\frac{D}{L} = 1{\cdot}45$) the proportions of apparent dextrose and laevulose are very nearly equal, as might be expected if they were formed from saccharose, but at night the dextrose appears to be in excess. A striking difference from Series II is that the dextrose instead of rising during the day and then falling, at first appears to *fall* and then to rise (see Fig. 3); at night, instead of a rise and fall, there is a fall between 9 and 11 p.m., followed by a gradual rise to 5 a.m. But in both series there appears to be a similar periodic character in so far as there are three rises and three falls in the 24 hours. In Series III the variation of the apparent dextrose is most striking as taking place along practically straight lines in a very regular manner. The apparent laevulose curve in Series III is entirely different from the dextrose curve but it follows fairly closely the curve of total hexoses, and less closely the curve of saccharose. The fluctuations of the apparent laevulose are considerably greater and more abrupt than those of the dextrose, as was also the case in Series II.

The curve showing the variation of $\frac{D}{L}$ is also given. $\frac{D}{L}$ falls from 9 a.m. to 1 p.m., then rises slowly and more or less by successive steps to a maximum at 11 p.m., when it again falls and rises twice before 9 a.m.

B. *Stalks and Mid-ribs.*

The most striking fact which appears from the data given in Tables IV to VII is that in the stalks and mid-ribs the apparent dextrose is always in large excess of the laevulose. Whereas in the *leaf* the ratio $\frac{D}{L}$ does not depart much from unity[1], in the mid-ribs and stalks it is rare to find this ratio anywhere in the neighbourhood of 1. Only in the earliest stages of growth (August 26th) and then only in the top half of the stalks, nearest the leaf, at noon and 6 p.m., when freshly formed sugars from the leaf are passing into the stalks, does the ratio

[1] The departure of this ratio from unity in Series I, Table I, is to be attributed to the impossibility, owing to the presence of optically active impurities, of ascertaining the true proportions of dextrose and laevulose in these cases, where the reducing sugars are present in small amounts. In Series II and III, the ratio $\frac{D}{L}$ is nearly unity throughout the whole 24 hours.

$\frac{D}{L}$ become nearly equal to unity. In all other cases the ratio varies from 2·5 to 5, or even higher in the case of the bottom halves of the stalks. In passing from the leaves to mid-ribs, from mid-ribs to the tops of stalks and from the tops of stalks to the bottom the proportion of apparent dextrose steadily and rapidly increases.

Series I. Tops and bottoms of stalks. The analyses given in Table IV show that the proportion of apparent dextrose to laevulose $\left(\frac{D}{L}\right)$ is far higher in the bottom half of the stalks than in the top half at the same time. At 6 a.m. of August 26th at first sight it would appear that during the night the laevulose practically disappears from both top and bottom halves of the stalk just as it appeared to do in the leaf (see Table I) during the night in the same series. Lindet observed a similar phenomenon in the case of the sugar beet and attributed the predominance of dextrose in the stalks to the laevulose being used more rapidly than the dextrose for purposes of tissue building. But that it is quite unsafe to rely upon the polarimetric data as affording any real index of the proportions of dextrose and laevulose actually present in the stalks is shown by the following considerations. The stalks stand out in striking contrast to the leaves as regards the extraordinary divergences between the results obtained for saccharose by the reduction method and by the method of double polarisation. In some cases, for example at 6 p.m., Table IV, the polarisation results for cane sugar in the bottom halves are 40 per cent. *higher* than the values obtained by reduction; and yet *at the same time* the tops give polarisation results which are 85 per cent. *low*. The following table (Table VIII) gives a comparison of the data obtained for cane sugar by the two methods (reduction and polarisation), showing that the divergence is very much greater in the stalks than in the leaves and much more variable in its nature. The fact that the *tops* may give by polarisation a large apparent deficiency of saccharose and the *bottoms* at the same time a large excess as compared with the reduction values (see the data at 12 noon and 6 p.m., Table VIII), or *vice versa* as at midnight when the relations are reversed, points to the presence *in the top and bottom halves of the stalk at different periods of the day of quite different impurities, with different and opposite rotatory powers* (substances as different as *d*- and *l*-glutamine or *d*- and *l*-asparagine).

A careful comparison of Table VIII with the curves showing the variation of apparent dextrose and laevulose in the stalks (Figs. 4

and 5) shows that *it is possible to correlate the wide variations in the differences* (Δ) *between the reduction and polarisation values for cane sugar with the variations in the apparent dextrose and laevulose*, a fact which points to their having a common origin, namely the presence of optically active impurities. Fig. 4 shows the variation of the apparent dextrose and laevulose in the *top* half of the stalks and also the curves for cane sugar and total hexoses. During the whole 24 hours the "dextrose" fluctuates only slightly—there is a slight rise and fall during

TABLE VIII.

Divergence of Results for Saccharose by Reduction and Polarisation in Mangold Stalks—Series I.

		Saccharose found				Polarisation results *high* by % (Δ)	
		Citric inversion		Invertase inversion			
		By reduction	By polarisation	By reduction	By polarisation	Citric inversion	Invertase inversion
6 a.m.	Tops ...	3·36 %	2·85 %	4·14 %	4·56 %	−15·2 %	+10·3 %
	Bottoms	3·47	3·60	3·89	3·60	+ 3·7	− 7·4
12 noon	Tops ...	4·52	0·08	4·26	0·40	−98·0	−90·4
	Bottoms	—	2·99	4·12	4·19	+ 9·14	+ 1·8
6 p.m.	Tops ...	4·14	0·64	3·92	0·61	−84·6	−84·5
	Bottoms	4·03	5·65	4·09	5·77	+40·3	+41·2
12 night	Tops ...	4·12	5·31	3·98	5·10	+29·1	+27·8
	Bottoms	—	—	4·15	3·18	—	−23·3

the day and a slight rise and fall at night. The saccharose also is nearly constant during the 24 hours. But the "apparent laevulose" varies enormously. Between 6 a.m. and noon this sugar increases from *nil* to 4·5 per cent., but from noon onwards falls along almost a straight line until the zero is again reached shortly after midnight. The important fact to be noted is that *while the apparent laevulose increases the differences* (Δ) *between the polarisation and reduction values of cane sugar become more and more negative* (change from + 10 to − 90 for invertase values, Table VIII), whilst when the apparent laevulose falls the values of Δ become more and more positive (− 84 per cent. at 6 p.m., + 28 at midnight). It may be noted that the curve of apparent

laevulose follows more or less closely the general course of the curve of total hexoses (calculated as invert sugar, from reduction values only). But there is no real significance in this because with the dextrose values apparently constant, the laevulose figures, which are also calculated from the same reduction values, necessarily follow the figures for total hexoses (at any moment $D + L$ = total hexoses).

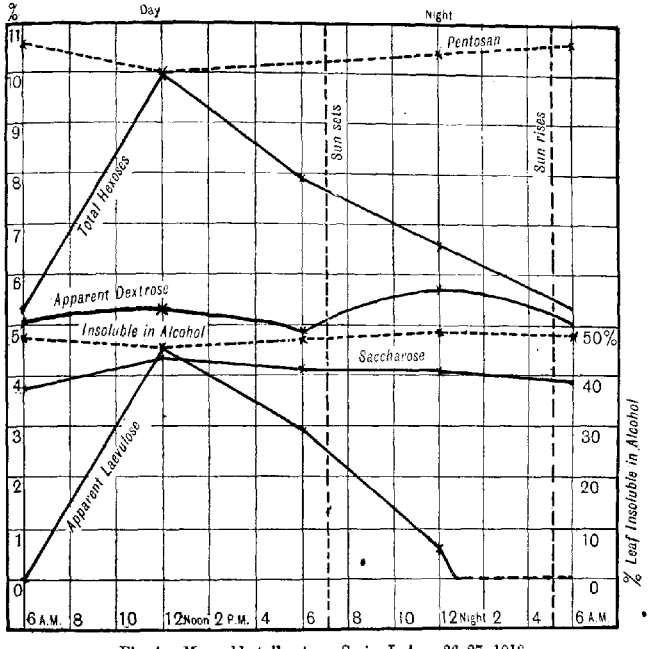

Fig. 4. Mangold stalks, tops, Series I, Aug. 26–27, 1913.

Exactly the same kind of relation can be traced between the fluctuation of the apparent hexoses and the values of Δ in the *bottom* halves of the stalks (Fig. 5). In this case, however, *both* the dextrose and laevulose appear to undergo wide variations. From 6 a.m. to noon the dextrose increases *absolutely* faster than the laevulose (from 8·55 to 11·0 per cent. for dextrose compared with a change from 0·2 to 1·8 per cent. for laevulose) although *relatively* the dextrose does not increase so rapidly as the laevulose, as shown by the fall of $\frac{D}{L}$ from 37·2 to 6·0.

The increase in the proportion of apparent dextrose is accompanied by the rise of Δ from a negative value (− 7·4, invertase figure, Table VIII) to a slightly positive value (+ 1·8). From noon to 6 p.m., although both dextrose and laevulose appear to be falling, the laevulose relatively

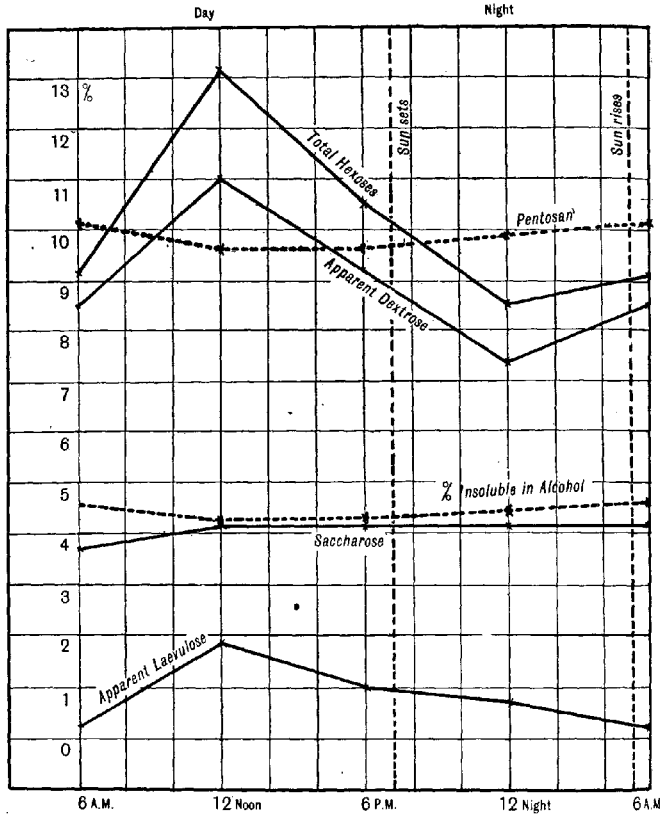

Fig. 5. Mangold stalks, bottoms, Series I, Aug. 26–27, 1913.

falls faster than the dextrose $\left(\frac{D}{L}\ \textit{increases}\ \text{from } 6{\cdot}0 \text{ to } 9{\cdot}1\right)$ and the value of Δ increases to + 41 (Table VIII). Between 6 p.m. and midnight laevulose appears to fall little, but the dextrose rapidly, at the same

time Δ changes from + 41 per cent. to a negative value − 23 per cent. After midnight the apparent dextrose suddenly begins to rise again and the laevulose to fall; as would be expected the value for Δ becomes less negative (changes from − 23 to − 7·4).

As showing the gradual transference of the optically active impurities from the tops to the bottoms of the stalks, it is interesting to compare the values in Table VIII for say 6 a.m. with those for 12 noon. The impurity in the 6 a.m. tops is such as to cause Δ to have a positive value + 10·3 per cent. (invertase); at the same time, however, the bottoms have a negative value − 7·4, but at 12 noon the value of Δ for the bottoms has become positive, viz. + 1·8 (the sum of + 10·3 and − 7·4 is + 2·9). Similarly at 6 p.m. the value of Δ is negative in the tops (− 84·5) but positive in the bottoms, but at midnight the bottoms show a negative value, − 23·3; had the whole of the material causing the negative value at 6 p.m. been transferred to the bottoms, the change expected would be − 84·5 + 41·2 or − 43·3.

In the case of the stalk bottoms (Fig. 5), where the fluctuation of the apparent laevulose is relatively small, it is the dextrose curve which follows most closely the curve of total hexoses, but as pointed out in the case of the laevulose in Fig. 4 this has no real significance and is a result merely of the method of calculation.

If one compares merely the relative position of the apparent dextrose and laevulose curves in Figs. 4 and 5, dextrose seems to accumulate at the bottoms of the stalks far more than the laevulose, the values for dextrose (7·4 to 11·00 per cent.) being higher in Fig. 5 than in Fig. 4 (4·87 to 5·71), whilst the fluctuations of laevulose are smaller (0·23 to 1·8 per cent. in Fig. 5 as compared with 0 to 4·5 per cent. in Fig. 4). But from the considerations already brought forward it is clearly quite unsafe to conclude that it is actually dextrose which accumulates at the bottom of the stalks, as large quantities of other optically active substances are undoubtedly present, which cause the wide divergences between the results for cane sugar by the polarisation and reduction methods. If dextrose were the principal sugar present (in some cases it appears to be, as at 6 a.m., the sole hexose in the stalks) it would point, as assumed by Lindet, to the laevulose being largely consumed on the way from leaf to root for constructive purposes; but it would necessitate also that the saccharose in the root is built up from the dextrose being conveyed to it. This would involve a transformation in the root of dextrose into laevulose, followed by a synthesis of cane sugar from dextrose and laevulose. Whilst this operation is a possible one, it is more

likely that the actual reducing sugars in the stalk reach the root as invert sugar[1] and that the apparent predominance of dextrose in the stalks is due solely to dextro-rotatory impurities; the existence of these is clearly proved by the enormous differences found in the cane sugar estimations by the polarisation method. Until really reliable methods of determining the true proportions of dextrose and laevulose have been devised it is impossible to draw any further conclusions on this point.

Series II. The considerations put forward above, correlating in Series I the apparent dextrose and laevulose values with the divergence between the cane sugar values determined by the reduction and polarisation methods, hold for the stalks and mid-ribs in Series II also[2]. The following table gives the values of Δ, and Figs. 9 and 10 of the preceding paper show the curves for dextrose and laevulose. It will be seen that, as in Series I, when the apparent laevulose increases rapidly as compared with dextrose the values of Δ become less positive; when the apparent laevulose decreases, the values become more and more negative.

Fig. 9 of the preceding paper shows the apparent variation of dextrose and laevulose in the stalks as compared with that of saccharose and the total hexoses and the variation of the ratio $\frac{D}{L}$. As in the tops of stalks in Series I the apparent dextrose rises slightly during the day (10 a.m. to 4 p.m.) but then remains practically constant until 4 a.m. next morning. The laevulose rises considerably more rapidly from 10 a.m.

[1] It is quite possible that the ratio of dextrose to laevulose in the mixture of sugars reaching the root is not strictly 1, owing to one of the sugars being put more under contribution for purposes of growth or respiration in the leaves or stalks than the other. But it is probable that the ratio is very nearly unity as is the case in the leaves (September and October), when the amount of optically active impurities interfering with the determination is a *minimum*.

[2] The same principle can be applied to the leaves of Series II and III to explain the fluctuations of Δ, i.e. the difference between the results found for cane sugar by double polarisation and by reduction, which are far less marked in the case of the leaves than with mid-ribs and stalks, because the proportion of optically active impurities is relatively less. In practically all cases when the apparent dextrose increases faster than the apparent laevulose, the divergence becomes increasingly positive; when the laevulose increases faster than dextrose the divergence becomes more negative. As pointed out on p. 339, when $\frac{D}{L}$ is unity there is the closest agreement between the results for cane sugar obtained by the two methods and the departure of the ratio $\frac{D}{L}$ from 1 is probably merely apparent and not real.

to 4 p.m. then falls until 11 p.m., when a second rise occurs. In the mid-ribs (Fig. 10 of preceding paper) the reverse is the case, the dextrose being nearly constant during the day and falling at night, whilst the laevulose falls by day and increases by night.

TABLE IX.

Divergence of Results for Saccharose in Stalks by Polarisation Method. Series II. September 10th–11th, 1912.

Time	% saccharose found				Polarisation results high by % (Δ)		$\frac{D}{L}$ (xylose)
	Citric inversion		Invertase inversion				
	Reduction	Polarisation	Reduction	Polarisation	Citric inversion	Invertase inversion	
10 a.m.	5·25	5·82	4·39	—	+10·7	—	3·33
4 p.m.	5·75	4·70	4·78	6·59	−17·9	+35·0	2·34
11 p.m.	5·18	8·26	—	6·67	+60·0	—	4·56
4 a.m.	5·34	5·38	5·10	6·45	+ 0·8	+26·5	3·30
6 a.m.	5·25	5·68	4·88	4·82	+ 8·4	+ 1·9	2·84

SUMMARY.

1. It is shown that in the extracts of mangold leaves and stalks optically active impurities are always present which are not precipitated by basic lead acetate and hence vitiate the estimation of the dextrose and laevulose. These substances are possibly acid amides (such as glutamine and asparagine) or amino-acids (such as glutamic and aspartic acids) which form soluble lead salts.

2. These impurities occur in the leaves, but are much more abundant in the mid-ribs and stalks.

3. In the leaves the dextrose and laevulose appear to be present in approximately equal amount, as would be expected if they were formed from saccharose by inversion. When the ratio $\frac{D}{L}$ departs from unity it is probably owing to the presence of a dextro-rotatory impurity (glutamine?) which increases the amount of dextrose apparently present; but at certain times of the day a laevo-rotatory impurity seems to predominate so that the ratio $\frac{D}{L}$ becomes less than unity.

4. In the mid-ribs and stalks, especially at the bottoms of the latter, the dextrose always appears to be in very large excess as compared with the laevulose; this is probably due to the proportion of the dextro-rotatory impurity being relatively greater in these parts than in the leaf, as is shown by the divergences between the polarisation and reduction values of saccharose being far greater.

5. The apparent fluctuations in the *ratio* of dextrose to laevulose are probably due to fluctuations in the optically active impurities rather than to variations in the sugars themselves. This is shown by the fact that these fluctuations can be correlated with the differences between the cane sugar values as determined by reduction and polarisation. When the apparent laevulose increases faster than the dextrose the results for cane sugar obtained by polarisation are *lower* than the reduction values; when the apparent dextrose increases faster than the laevulose or the laevulose falls more rapidly than the dextrose, the polarisation results are in excess of the true values.

6. The fluctuations of the apparent dextrose and apparent laevulose take place more or less regularly during the 24 hours; this points to a regular variation in the optically active impurities.

7. In the *leaves* the values of saccharose obtained by the double polarisation method are almost always *higher* than the reduction values; in the stalks, however, they are sometimes very high and sometimes very low. This is probably due to the presence of at least two different optically active substances at different times of the day. The increase of the apparent laevulose corresponds with the increase of the substance causing low results for cane sugar by the double polarisation method; the increase of apparent dextrose corresponds with a falling off of this substance and the formation of the impurity which gives high results.

8. Until more reliable results can be obtained for the true dextrose and laevulose by methods which are independent of the polarimetric data, it seems justifiable, from the results brought forward, to assume that the dextrose and laevulose exist in the leaves and stalks as invert sugar and travel in nearly, if not exactly, equal proportions to the root, where retransformation into saccharose occurs. This assumption best agrees with the regular rise and fall of the total hexoses in the stalks and mid-ribs along almost straight lines during the night, as contrasted with the more irregular fluctuation of the apparent dextrose and laevulose taken separately.

9. It is impossible in the present state of our knowledge to draw any conclusions from the proportion of apparent dextrose or laevulose

in plant tissues as to whether either of these sugars is better adapted than the other to tissue formation or to respiration. All such conclusions in the past are valueless because the analytical methods at present existing do not give true values for these sugars.

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STUDIES OF THE FORMATION AND TRANSLOCATION OF CARBOHYDRATES IN PLANTS.

III. THE CARBOHYDRATES OF THE LEAF AND LEAF STALKS OF THE POTATO. THE MECHANISM OF THE DEGRADATION OF STARCH IN THE LEAF.

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(*Rothamsted Experimental Station.*)

In the first two papers of this series (pp. 255–351) we have dealt with the carbohydrates of the mangold, a plant which, like its near relation the sugar beet, stores only saccharose in its root. One of the most striking features of this plant is that it forms no starch in the leaf, except during the very earliest stages of growth when it is a seedling; it is only during this period, when the root is very small and has not developed sufficiently to store the sugars formed, that starch appears in the leaf at all. When the mangold has begun to develop a large storage reservoir in the root and the sugar can be readily translocated away so that all danger is avoided of too high a concentration in the leaf, starch ceases to be produced, and during the whole of the growth in August, September, and until the roots are lifted at the end of October, it is entirely absent from the leaf. Maltose too is entirely absent. In these respects the mangold, although a dicotyledon, resembles monocotyledonous plants such as the onion (*Allium cepa*) and snowdrop (*Galanthus nivalis*), which do not form starch in the leaf although they store both starch and inulin in the bulb; in many respects, as we have shown, the phenomena of formation and translocation of sugars in the mangold are similar to those observed by Parkin [1912] in the

[1] Mr A. J. Daish, who shared our earlier work, would have taken part in this investigation had not his military duties, after the outbreak of war, rendered it impossible. He assisted us during the heavy work of the 24 hours picking of July 16–17th, 1914, and we wish here duly to acknowledge this.

snowdrop. It appears, however, that in the later stages of growth (September and October) certain gummy substances, which were not studied in any detail, are formed as a reserve in the leaf tissue and appear to be broken down to sugars at night, thus playing a similar part to the starch in most foliage leaves.

In view of the fact that we found, by using our method of estimating maltose by maltase-free yeasts, that maltose is entirely absent not only from the leaf and stalks of the mangold, which does not store starch, but also from the leaves of many other plants which form an abundance of starch, we considered it desirable, in order to test Brown and Morris' views [1893] as to the part played by diastase during the night in breaking down the starch to maltose, to study the variation of the carbohydrates in the potato leaf throughout a complete 24 hours period. The potato forms considerable quantities of starch in its leaf and if, as seemed possible, maltose is an intermediate stage in the synthesis of starch, just as it is in its degradation by enzymes, it should appear in the leaf, at least in small quantities, during the day; if the starch is broken down by ordinary diastase in the way suggested by Brown and Morris, maltose should appear in increasing quantities at night during the disappearance of the starch from the leaf. Finally, if Brown and Morris' view [1893, p. 673] is correct that maltose is the translocation form of starch, maltose should be found in the stalks.

Our experiments (see Tables I and II, p. 366) show that maltose is entirely absent from the leaf and stalks of the potato at all periods of the day and night. We have now made nearly 500 analyses by means of maltase-free yeasts of many different kinds of plants, including the nasturtium (*Tropæolum majus*), turnip, carrot, sunflower (*Helianthus annuus*), dahlia, *Arum maculatum* and vine (*Vitis vinifera*); *in no case has maltose been found either in the leaf or stalks*, even in such plants as the turnip or nasturtium which store very large quantities of starch in the leaf[1]. We need here only refer to the data given in a previous paper (Davis and Sawyer [1914]) for the quantitative fermentations carried out with the alcoholic extract of the turnip leaf (starch = 12·77 per cent. of the total vacuum-dried leaf). In order to work with as

[1] In one case (July 9th, 1913) the leaf of the turnip was found to contain 18·73 per cent. of starch calculated on the vacuum-dried matter left after extracting the sugars, etc., with alcohol; this calculated on the total vacuum-dried matter of the leaf, including the sugars, becomes 12·79 per cent. In a sample of *Tropæolum* leaf (July 4th, 1913) the starch formed 26·75 per cent. of the dry leaf after extraction, or 17·6 per cent. of the total vacuum-dried matter of the original leaf.

large a quantity of substance as possible 1 litre of the purified aqueous solution of the sugars (a quantity which represented 44·69 grms. of the total vacuum-dried leaf matter) was evaporated *in vacuo* to 175 cc. and made up to 250 cc. Three portions of 50 cc. each were fermented during 3 weeks to 1 month with *S. marxianus* and *S. exiguus*, and after treatment with alumina cream made up to 100 cc. Two duplicate fermentations were carried out with a pure culture of distillery yeast.

50 cc. of the filtrate (representing 4·469 grms. of T.V.D.M.) gave in the case of the *S. marxianus* and *S. exiguus*, 0·0524 grm. CuO; and gave in the case of the distillery yeast, 0·0500 grm. CuO.

These values are practically identical and well within the range of error of the method. They show that *maltose was entirely absent from the turnip leaf* in question. The reducing power, as pointed out [1914], is to be attributed to unfermentable *pentoses*; it corresponds with 0·51 per cent. of pentose; 0·60 per cent. was found by the ordinary phloroglucinol method when applied directly to the solution containing the sugars, prior to fermentation.

Brown and Morris, in their important memoir, gave what was undoubtedly good evidence of the presence of maltose in their extracts of *Tropæolum* leaf. They were not content merely with the analytical data but endeavoured "in view of the immense importance which must necessarily be attached to this product of starch hydrolysis to obtain more direct evidence of its presence." They succeeded in isolating an osazone, apparently identical with maltosazone, from the solution of the mixed sugars contained in a large quantity of the dry leaves of *Tropæolum*, and analysed it. Finally they showed the presence of maltose by treating a solution of the mixed sugars of the leaf, after completely inverting the saccharose, with a preparation of the enzyme maltase. This enzyme always brought about a large increase in the cupric reducing power, amounting generally to about 75 per cent. of the increase observed on digesting the same solution with dilute acid.

It is impossible in view of these facts to doubt that maltose was present in the material worked with by Brown and Morris. It is, however, possible to reconcile these results with our own by taking into account the difference between the methods of extraction of the sugars adopted by Brown and Morris and by ourselves. We have been led to conclude that plant leaves which store starch contain in addition to the enzymes of ordinary diastase (a fact which was first definitely proved by Brown and Morris) the enzyme *maltase*, which is capable of breaking down maltose to dextrose. We have shown in a previous paper

(Davis and Daish [1914]) that taka-diastase, the mixture of enzymes isolated from *Aspergillus oryzae,* differs from the ordinary diastase of malt extract mainly in containing maltase in addition to the ordinary starch resolving enzymes. Taka-diastase therefore converts starch paste completely into a mixture of maltose and dextrose, the latter rapidly increasing in amount until 80–85 per cent. of the sugar is in this form. We consider that the ordinary foliage leaf contains a mixture of enzymes similar to that elaborated by *Aspergillus oryzae*, and of such a nature that the maltase is always present in relative excess so that the maltose formed by the breaking down of the starch is very rapidly and completely converted into dextrose. Now in our method of preparing the leaf samples for analysis, the material was dropped into boiling alcohol containing a little ammonia, so that the enzymes were destroyed instantly, but Brown and Morris and most other workers in this field *dried their leaf material in an oven before extracting the sugars.* During this drying, owing to the large quantity of moisture in the leaf, the temperature only rises gradually and the enzymes continue to act for a considerable time before they are destroyed. Maltase is the first of the enzymes to be put out of action; it is well known to be one of the most unstable of enzymes. Our experiments (Davis, 1914, 1) with taka-diastase show that it is largely destroyed before a temperature of 55° is reached. When leaves are dried in an oven, after the maltase has been destroyed at say 50° C., the ordinary diastatic enzymes continue to act under optimum conditions as regards temperature and considerable quantities of starch are broken down to dextrin and maltose. This action lasts until the temperature rises to about 80°, when the dextrin and maltose-forming enzymes are also destroyed. As the maltase has been completely killed in the earlier period of drying, the maltose formed in this way will persist as such and be found in the mixture of sugars subsequently extracted from the dried material.

In support of this explanation of the differences between Brown and Morris' results and our own, several facts may be adduced. In Brown and Morris' experiments the proportion of starch in the freshly plucked nasturtium leaf at the end of a sunny day was found to range between 2·9 and 7·4 per cent. of the total dry matter; we have always found the starch in the same leaf to be considerably higher, thus in the example given on p. 353, footnote, the starch was 17·6 per cent. of the total vacuum-dried matter or about two and a half times the highest figure given by Brown and Morris. In one case cited by these workers the starch was found to be 4·59 and the maltose 5·33 per cent., the sum of the two being

9·9 per cent.; this is still considerably lower than the values we have found. Kluyver [1914] recently on repeating Brown and Morris' experiments with *Tropœolum*, but using a new biochemical method of estimating the hexoses, saccharose and maltose[1], based on the differences in the amount of carbon dioxide evolved on fermenting the solution with special *torulae* and with ordinary yeast, also found in all cases relatively very small amounts of maltose. Thus in one case, which is of special interest because the hexoses and cane sugar are nearly identical with values found by Brown and Morris, Kluyver found in leaves plucked at 2.30 in the afternoon, hexoses = 5·2 per cent., saccharose = 4·6 per cent., *maltose* = 0·3 per cent.; this compares with Brown and Morris' analysis, hexoses = 5·6 per cent., saccharose = 4·9 per cent., *maltose* = 1·2 per cent. In the remaining cases which Kluyver cites the results are given merely relatively, without calculating back on the dry matter of the leaf: but, in every instance, the proportion of maltose found was exceedingly small as compared with the saccharose and hexoses. Thus, for example, a sample of *Tropœolum* leaf picked at 4 p.m. on July 28th, and therefore corresponding with an analysis in which Brown and Morris found saccharose 8·02 per cent., maltose 3·62 per cent., and in which maltose formed 27·5 per cent. of the total sugars, gave

Saccharose	...	25·8 mgrms.
Hexoses	...	21·8 ,,
Maltose	...	1·6 ,,

The maltose found here forms only 3·2 per cent. of the total sugars. In Brown and Morris' experiments the maltose always appeared to be at a maximum at the end of the afternoon, that is at the same time as the starch reached its highest values; for example, in one case (p. 669) a leaf picked at 5 p.m. when the starch formed 4·59 per cent. of the dry leaf, maltose was found to be present to the extent of 5·33 per cent., *and to form* 56 *per cent. of the total sugars.*

These results are fundamentally different from Kluyver's obtained at the same time of day and the difference is probably to be explained

[1] *Torula monosa* does not contain the enzymes maltase and invertase, and hence is capable of fermenting the hexoses only, leaving the maltose and saccharose unchanged; *Torula dattila* contains invertase but not maltase, and therefore ferments cane sugar and the hexoses but not maltose. Dr A. J. Kluyver has been kind enough to send us pure cultures of these Torulae, which Dr H. Limbosch has tested for us in our laboratory, according to our own methods of working, on very carefully purified specimens of sugars. We can confirm Kluyver's statements as to the specific nature of these organisms which should prove of considerable service in sugar analysis of the kind we have had to deal with.

by the fact that in Brown and Morris' experiments the heating up of the leaves in drying was much slower and allowed far more diastatic action to occur than in Kluyver's experiments. Kluyver especially points out that his leaves, which were dried in thin layers in a baker's oven heated to 105°, were exposed to the drying process during only 5 to 10 minutes. We have ourselves made several experiments with *Tropæolum* leaves dried *rapidly* in a steam oven and by our own methods have invariably found *no* maltose to be present, just as in the case of the same leaves dropped into boiling alcohol.

From the above facts we have concluded that the maltose which was undoubtedly present in Brown and Morris' experiments in relatively large amounts and in Kluyver's experiments in far smaller proportions owing to the greater rapidity of drying, was not formed in the tissue of the leaf as such during growth, but was produced by the degradation of starch by the diastatic enzymes remaining after the maltase in the leaf had been destroyed in the first stage of the drying process. As regards the mechanism by which starch is utilised in the plant when, at the end of the day, the reserves in the leaf are called upon, *we consider that the starch is hydrolysed completely to dextrose by the leaf enzymes*, which resemble the enzymes of *Aspergillus oryzae* in containing an abundance of maltase. Brown and Morris' main view that the starch is utilised by a purely enzymic process seems to us perfectly correct, but we regard the enzymic degradation as stopping, not at maltose, as supposed by Brown and Morris, but at the stage of dextrose, the final product of starch hydrolysis. One of us has shown (Davis, *Chemical World*, 1914, p. 271) that yeasts which do not contain the enzyme maltase, for example, *S. anomalus* and *S. exiguus*, are quite unable, even when in the throes of starvation, to make use of maltose in the solution in which they are growing; similarly we find that *Torula monosa*, which does not contain invertase, is unable to make use even of cane sugar. Plant tissue, we consider, in exactly the same way before it can utilise starch, maltose or saccharose, for purposes of growth, must break these substances down to the simple hexoses by enzyme action. This view explains the significance of the fact that the sugars in the stalks of all the plants we have examined consist largely of the simple hexoses; these sugars are capable of being directly assimilated by the cambium layer of the stems or by other growing points. The necessity of transformation of saccharose into invert sugar thus explains the almost ubiquitous presence of invertase in the plant, except in such storage reservoirs as the mangold root, where cane sugar is permanently housed.

The views we put forward are in accord with modern views, based largely on the work of Abderhalden and his school, as to food assimilation by animals; in all cases it is necessary for such food, for example, proteins, to be broken down by enzymes into its simplest components or "*Bausteine*," which are then taken up by the different cells or tissues and synthesised afresh.

The theory we have given of the method by which starch is broken down in the leaf would lack justification unless definite evidence of the presence of maltase in leaf tissue could be brought forward. At the suggestion of one of us, Mr A. J. Daish has made a special study of this question. In a series of experiments, details of which will be published later, he has found that maltase is always present in the leaf tissue he has examined when starch is also present. Little doubt therefore can be entertained of the correctness of the view we put forward that starch is broken down in the leaf to dextrose. The fact that maltose can never be detected either in the leaf or stalks of plants points to the amount of maltase always being in relative excess in the cells where the starch degradation actually occurs, so that it is able to deal instantly with the whole of the maltose formed from the starch. The fact that maltose, unlike cane sugar, never occurs in the stalks or conducting vessels is probably due to the fact that maltase is an intracellular enzyme and apparently acts in close collaboration and in the immediate proximity of the ordinary diastase which first attacks the starch in the cells where this substance is stored.

Cane sugar is apparently the first sugar formed in the potato leaf and is transformed into hexoses for translocation.

The most striking point which appears when the analyses of the potato leaves and potato stalks are compared (see Tables I and II) is that whereas *the saccharose is greatly in excess of the hexoses in the leaf, the reverse is true in the stalks.* These results are exactly similar to those obtained with the mangold leaf in the early stages of growth (Series I), a fact which points to the mechanism of formation and translocation being the same in both cases. Saccharose is probably the first sugar formed in the mesophyll of the leaf; it is gradually inverted on its way through the veins, mid-ribs, and stalks, the inversion becoming more and more complete as the root or tuber is approached. In this series of pickings it must be borne in mind that the "stalks" were mainly those bearing the small leaflets and did not include any of the stem

in the neighbourhood of the tuber where, by analogy with the mangold and snowdrop (Parkin [1912]), the hexoses would be found probably to preponderate even more than is shown in Table II. Time has allowed us only to take one series of pickings with the potato, but it seems highly probable that, as in the case of the mangold, sugar beet, and snowdrop, the proportion of hexoses to saccharose becomes greater and greater in both leaf and stalk as the season advances, and the storage function becomes more and more predominant.

As regards the transformation of the hexoses into starch in the tuber, it is interesting to note that in this way the hexoses are as it were imprisoned and held until required for later use, when the appropriate enzymes again degrade the starch to sugars. In the mangold the imprisonment of the hexoses in the root is effected by their transformation into cane sugar.

From data which we have obtained with many other plants, to be published later, it appears that cane sugar is produced, generally in a predominant proportion, in the leaf of *all* plants, whatever be the form in which the sugars are finally stored (cane sugar, starch, inulin or dextrose). Thus, for example, we find that, when proper precautions are taken to prevent enzymic change, contrary to Deleano's [1912] recent statement, cane sugar is the principal sugar of the vine leaf (*Vitis vinifera*). In this plant the storage form is dextrose, and unless the cane sugar is a primary product of the mesophyll tissue it is difficult to see any special reason for its predominance in the leaf. If dextrose and dextrose alone were, according to Strakosch's [1907] views, the direct product of photosynthesis, one would expect to find it the principal if not the sole sugar in the leaf of a plant which stores dextrose as its reserve carbohydrate. In fact, as stated in our previous paper (I), all the data we have obtained with plants of many different kinds best harmonise with the view put forward by Brown and Morris [1893], that saccharose is the first sugar formed in photosynthesis and that the hexoses are formed from it and not *vice versa*. It seems to be the general *function of the mesophyll tissue to elaborate saccharose directly*; this is broken down in the veins, mid-ribs and stalks, and reaches the place of storage in the form of hexoses. Unless saccharose is a primary product it is difficult to see why it should predominate in the leaves of plants of such different types as the potato, the vine, sunflower and snowdrop, in none of which is cane sugar the storage form; there seems, indeed, no useful purpose in its production at all in such cases, as the substances stored are undoubtedly built up from hexoses, which are the

predominating constituents of the sap in the *stalks*, and could very well be translocated directly as such from the leaf. It is possible, and may be argued, that the saccharose in the leaf serves to regulate the osmotic pressure, owing to the ready interconversion of saccharose and hexoses; but in plants which form starch, such as the potato, this regulation could be quite as well effected by the precipitation of the polysaccharide and the function of the cane sugar is not easily understood unless it be regarded as a primary and compulsory product of the mesophyll.

The Dextrose-Laevulose Ratio. As was the case in the mangold leaf, it is shown that it is impossible to obtain accurate values for dextrose and laevulose owing to the presence in the solutions of optically active impurities which are not removed by the ordinary process of defecation by basic lead acetate. These impurities also interfere with the estimation of the saccharose by the double polarisation method and, as in the mangold, the fluctuations of the apparent dextrose and laevulose can be correlated with the divergences between the values found for saccharose by the reduction and by the optical methods (see p. 344). It appears that *two* optically active impurities with rotations of opposite sign are formed at different periods of the 24 hours, and it is the variation of these that causes the apparent fluctuations in the proportion of dextrose and laevulose. In the leaf a substance with a laevo-rotatory power generally predominates, so that the laevulose appears to be greatly in excess of the dextrose; but in the stalks this is no longer the case and dextrose appears to be largely in excess of the laevulose.

Experimental.

The methods of sampling, extraction, and analysis were the same as those described in the case of the mangold (see Paper I). The potatoes (King Edward VII) were grown on a piece of ground at the side of the laboratory; at the date of picking (July 16th–17th, 1914) the plants were just beginning to form flower buds and the tubers were small. Rain had fallen heavily on July 12th, but the days following were dry and sunny. Pickings were taken every two hours. The leaflets were detached from the rachis, but the mid-ribs of these leaflets were not cut out so that the results given for "leaves" refer to the whole leaflets including these mid-ribs; what we have called "stalk" consisted in reality mainly of the rachis of the compound leaves and included only a small portion of the main stalk or stem, namely the portion furthest from the tubers.

Estimation of Starch and "Soluble Starch."

The dried potato leaf obtained after completely extracting the sugars and other substances soluble in 80 per cent. alcohol was found to contain large quantities of a substance readily soluble in water and possessing a high positive rotation. This made it necessary to modify the method of estimating starch which we have employed (Davis and Daish [1914]) by first completely extracting this substance with water from the portion of material used in the analysis. At certain times of the day (4 p.m. to 8 p.m.) the aqueous extract so obtained contained a substance which resembled soluble starch or dextrin in yielding a mixture of maltose and dextrose on treatment with taka-diastase. In all cases the reducing power (if any) and rotatory power of the aqueous extract were determined after diluting to a known volume (250 cc.); an aliquot portion (150 cc.) was then treated with taka-diastase, and, after the conversion, with basic lead acetate (which generally produced a copious precipitate owing to the presence of tannins, etc.), being then diluted to a known volume (200 cc.). The reducing and rotatory powers of the solution were determined and from the change in these brought about by the taka-diastase the "soluble starch" (or dextrin) was calculated. In most cases the "soluble starch" was *nil*, but between 4 p.m. and 8 p.m. considerable quantities could be detected. Even in these cases, however, the amount of soluble starch found in this way did not account for more than 25 to 50 per cent. of the rotation observed in the aqueous extract; in all cases, too, the basic lead acetate added after the conversion produced a heavy, gelatinous precipitate, pointing to the presence of tannins, gums, etc. The aqueous extract before conversion invariably had a slight cupric reducing power (50 cc. of the 250 cc. gave 0·01 to 0·02 grm. CuO) which may perhaps have been due to unextracted sugars; but as in the experiments with mangold leaves, the extraction of sugars was always complete, it is probable that the reduction was due to a substance of the tannin class. For purposes of comparison we give in Table I the actual values calculated for the rotation $(\alpha)_D$ in a 200 mm. tube of the aqueous extract of the leaf material corresponding with 100 grms. of the *total vacuum-dried matter* of the leaf (including the sugars and alcohol soluble substances). The value is also given for the "soluble starch" ($[\alpha]_D = 202°$) that this would correspond with, calculated as a percentage on the total vacuum-dried matter. Thus a comparison can be made of the true soluble

starch found by the taka-diastase and the value found in this way from the rotation.

The true *starch* in the leaf was estimated by treating with taka-diastase the leaf material remaining after extraction with water; this was, of course, first gelatinised by boiling water in the usual way.

The following is an actual example showing the method of analysis and calculation.

Potato Leaf, 8 p.m., July 16th, 1914.

10·7148 grms. of the dried powdered leaf material remaining after the extraction of sugars[1], dried at 110° *in vacuo* until the weight was constant gave 9·8405 grms.; the moisture present therefore = **8·16** per cent.

The vacuum-dried weight of matter soluble in alcohol for this picking was 59·25 grms.

The weight of oven-dried matter (fibre, starch), etc., left after extraction with alcohol was 111·55 grms.; as the moisture in this was 8·16 per cent., the vacuum-dried weight = 102·42 grms.

The total vacuum-dried matter (T.V.D.M.) therefore

$$= 102{\cdot}42 + 59{\cdot}25 \text{ grms.} = \mathbf{161{\cdot}67} \text{ grms.}$$

The 9·8405 grms. of leaf substance was transferred to a beaker flask of 250 cc. capacity and left with about 200 cc. of water and 2 cc. of toluene for 24 hours at 38°, stirring well at intervals. The clear solution was then decanted through a starch-free filter paper as completely as possible and the residue washed by decantation until the volume in the flask was 250 cc.

Aqueous extract. 50 cc. of the 250 cc. gave 0·0217 grm. CuO.

Polarisation in 200 mm. tube = + 0·358° (sodium flame, 20°). This rotation calculated as 100 grms. of vacuum-dried extracted leaf

$$= \frac{0{\cdot}358 \times 2{\cdot}5 \times 100}{9{\cdot}8405} = 9{\cdot}10°.$$

Calculated on 100 grms. of T.V.D.M. $= \dfrac{9{\cdot}10 \times 102{\cdot}42}{161{\cdot}67} = \mathbf{5{\cdot}76}°$.

Calculated as soluble starch ($[\alpha]_D = 202°$) per 100 grms. of T.V.D.M.

$$= \frac{0{\cdot}358 \times 2{\cdot}5 \times 10^4 \times 102{\cdot}42}{2 \times 202 \times 9{\cdot}8405 \times 161{\cdot}67} = \mathbf{1{\cdot}43} \text{ per cent.}$$

[1] This had been dried in an oven at 100°, ground in a mill and kept in a stoppered bottle until the analysis was made. For precautions in sampling this material see Davis and Daish [1914], p. 161.

"Soluble Starch" (or dextrin) in Aqueous Extract.

150 cc. of the 250 cc. were left with 0·1 grm. of taka-diastase and 1 cc. of toluene for 24 hours at 38°; to the solution 5 cc. of basic lead acetate solution were then added, which was *just* sufficient to precipitate the whole of the tannins, gums, etc. The solution was diluted to 200 cc. at 15° and filtered; the slight excess of lead in the filtrate was *exactly* precipitated by adding solid sodium carbonate and the reducing and rotatory powers of the filtrate determined.

50 cc. of the 200 cc. gave 0·0718 grm. CuO.
Rotation in 400 mm. tube at 20° = + 0·202°.

Correcting for 0·1 grm. taka-diastase, under exactly similar conditions (correction for CuO = 0·0360 grm.; for polarisation = + 0·106°), we have

CuO due to sugars present = 0·0358 grm.
Polarisation due to sugars present = + 0·096°.

It is necessary to correct for the reducing power and polarisation of the original solution; for the reducing power we have

$$\frac{150}{200} \times 0{\cdot}0217 = 0{\cdot}0163 \text{ grm.}$$

As to the rotatory power, measurements made with the various pickings in which "soluble starch" was entirely absent showed that if the reducing substances be assumed to be sugars, with a cupric reducing power 2·5 grms. CuO per grm., they had the specific rotatory power $[\alpha]_D^{20°} = + 25°$. The assumption that this is the case when the soluble starch is present will give no sensible error; we have therefore α_D due to these substances in a 400 mm. tube

$$= \frac{0{\cdot}0163}{2{\cdot}5} \times \frac{25 \times 400 \times 2}{10^4} = + 0{\cdot}013°.$$

We have therefore as final values for maltose and dextrose formed by the diastase conversion:

CuO ex 50 cc. = 0·0358 − 0·0163 = 0·0195 grm.
Polarisation in 400 mm. tube = 0·096 − 0·013 = 0·083°.

If x = dextrose in 50 cc.; y = maltose in 50 cc.,

$$2{\cdot}58x + 1{\cdot}38y = 0{\cdot}0195$$
$$4{\cdot}22x + 11{\cdot}01y = 0{\cdot}0830$$

Solving, $x = 0{\cdot}00413$ grm.; $y = 0{\cdot}00586$ grm.

The total dextrose corresponding with leaf material taken

$$= 0{\cdot}00443 \times \frac{200}{50} \times \frac{250}{150} = 0{\cdot}0296 \text{ grm.}$$

The total maltose corresponding with leaf material taken

$$= 0{\cdot}00586 \times \frac{200}{50} \times \frac{250}{150} = 0{\cdot}0391 \text{ grm.}$$

"Soluble starch" corresponding with dextrose

$$= 0{\cdot}0296 \times 0{\cdot}9 \quad = 0{\cdot}02664 \text{ grm.}$$

"Soluble starch" corresponding with maltose

$$= 0{\cdot}0391 \div 1{\cdot}055 = 0{\cdot}0371 \text{ ,,}$$

$$\text{Total} \quad = 0{\cdot}0637 \text{ ,,}$$

Percentage of "soluble starch" $= \frac{0{\cdot}0637}{9{\cdot}8405} \times 100 = 0{\cdot}65.$

∴ Percentage of soluble starch on T.V.D.M. in leaf

$$= \frac{0{\cdot}65 \times 102{\cdot}42}{161{\cdot}67} = \mathbf{0{\cdot}41}.$$

True starch. The leaf material remaining after the extraction with water was gelatinised by heating with about 200 cc. of water during 30 minutes in boiling water; after cooling, 0·1 grm. taka-diastase was added and 2 cc. of toluene and the mixture left 24 hours at 38°, stirring at intervals. Two drops of concentrated sodium hydroxide were then added to destroy the enzyme and the solution filtered from the leaf material on a Buchner funnel; this was thoroughly washed with water by decantation until the total volume of the filtrate was about 475 cc. Basic lead acetate was then added (2·5 cc. was generally just sufficient) and the volume made up to 500 cc. The slight excess of lead in the filtrate was removed by adding *exactly* the necessary quantity of solid sodium carbonate and after filtering the reducing power and rotation of the solution were determined.

50 cc. gave 0·0908 grm. CuO

Polarisation = 0·264° in 400 mm. tube at 20°.

These values corrected for 0·1 grm. of taka-diastase under exactly similar conditions (CuO correction = 0·0138 grm.; polarisation = + 0·073°) give

Corrected CuO from 50 cc. = 0·0770 grm.,

Corrected polarisation = + 0·191°.

If x = dextrose in 50 cc.; y = maltose in 50 cc.,

$$2{\cdot}58x + 1{\cdot}38y = 0{\cdot}0770$$

$$4{\cdot}22x + 11{\cdot}01y = 0{\cdot}191°.$$

Solving, $x = 0{\cdot}02587$ grm. in 50 cc.

$y = 0{\cdot}00746$ grm. in 50 cc.

∴ Total dextrose in 500 cc. = 0·2587 grm. = 0·2328 grm. starch.

Total maltose in 500 cc. = 0·0746 grm. = 0·0707 „ „

∴ Total starch = 0·3035 grm.

Percentage of starch in the vacuum-dried extracted leaf

$$= \frac{0{\cdot}3035 \times 100}{9{\cdot}8405} = 3{\cdot}08.$$

Percentage of starch in the total vacuum-dried matter

$$= \frac{3{\cdot}08 \times 102{\cdot}42}{161{\cdot}67} = \mathbf{1{\cdot}95}.$$

SUMMARY OF ANALYSIS[1].

Rotation of aqueous extract calculated on 100 grms. T.V.D.M. in 100 cc.	= 5·76°
This represents as "soluble starch"	= 1·43 %
Actual "soluble starch" found by diastase	= 0·41 %
True starch found by diastase	= 1·95 %

[1] A duplicate analysis of this sample gave: Rotation of aqueous extract per 100 grms. T.V.D.M. = 5·58°; calculated as soluble starch this = 1·38 per cent. "Soluble starch" by diastase = 0·30 per cent.; true starch = 1·24 per cent. The duplicates here given for the true starch are not so close as is usually the case in these analyses; a more typical case (4 a.m.) gave 1·24 and 1·43 per cent. The greatest difficulty is encountered in the sampling, as was pointed out in a former paper; for each analysis the whole of the powdered leaf material, especially that at the bottom of the bottle, where the heavy starch grains tend to collect, should be turned out on a sheet of paper and 10 grms. sampled so as to represent a fair average of the whole of the material.

Results of Analyses.

Table I.

Potato Leaves, July 16th–17th, 1914.

July 16th. Sun rises 4.2 a.m. July 17th. Sun rises 4.4 a.m.
Sun sets 8.10 p.m.

Time	Temp. ° F.	% T.V.D.M. soluble in alcohol	Saccharose % on T.V.D.M.				Hexoses as invert sugar %	I.S. / C.S.	Pentose %	Pentosan %	Maltose %	Aqueous extract		Soluble starch %	True starch %	Remarks
			Citric acid	Invertase	Δ = C.A. − I.	Average						α_D per 100 grms. T.V.D.M.	α_D calc. as sol. starch %			
6 a.m.	57	37·2	2·16	2·11	+0·05	2·14	0·40	0·19	0·35	5·72	0·00	6·02°	1·49	0·00	1·88	Sunny
8 a.m.	60	39·1	2·47	2·60	−0·13	2·53	1·00	0·39	0·37	5·37	,,	8·32	2·06	,,	2·00	Sunny
10 a.m.	61	38·5	2·81	2·65	+0·16	2·73	0·37	0·14	0·52	5·30	,,	3·36	0·83	,,	2·55	Slightly overc
12 noon	62	39·3	3·39	3·19	+0·20	3·29	1·21	0·37	0·43	5·35	,,	5·06	1·24	,,	1·40	Shower 1.30
2 p.m.	63	34·1	3·81	3·50	+0·31	3·66	0·67	0·18	0·44	5·40	,,	5·30	1·31	,,	1·81	Shower 3.30
4 p.m.	63	36·2	3·56	3·34	+0·22	3·45	0·93	0·27	0·42	5·33	,,	8·88	2·20	0·58	1·56	Very bright
6 p.m.	64	35·8	3·46	3·22	+0·24	3·34	1·27	0·38	0·46	5·42	,,	8·96	2·22	1·00	5·95	Bright
8 p.m.	60	36·6	2·77	2·69	+0·08	2·73	1·22	0·45	0·42	5·51	,,	5·67	1·40	0·36	1·61	
																Dark 9.15 p.m
10 p.m.	59	34·8	2·76	2·49	+0·27	2·63	0·40	0·15	0·45	5·35	0·00	5·02	1·24	0·00	2·60	
12 night	56	36·6	2·48	2·30	+0·18	2·39	0·73	0·30	0·44	5·60	,,	8·32	2·05	,,	0·24	
2 a.m.	55	37·5	2·38	2·26	+0·12	2·32	0·68	0·29	0·37	5·74	,,	7·59	1·88	,,	0·28	
																1st light 3 a.m
4 a.m.	53	37·7	2·09	1·44 (?)	+0·65 (?)	1·76	0·15	0·08	0·43	5·70	0·00	5·71	1·42	0·00	1·33	

Table II.

Potato Stalks. July 16th–17th, 1914.

Time	Sugars in leaf %		% of stalk soluble in alcohol	Saccharose % on T.V.D.M.				Hexoses as invert sugar %	I.S. / C.S.	Pentose %	Pentosan %	Maltose %	Aqueous extract		Soluble starch %	True starch %	
	Saccharose	Hexoses		Citric acid	Invertase	Δ = C.A. − I.	Average						α_D per 100 grms. T.V.D.M. in 100 cc.	α_D calculated as soluble starch %			
6 a.m.	2·14	0·40	35·9	3·20	3·28	−0·08	3·24	4·94	1·52 / 0·43	0·43	12·45	0·00	8·93°	2·21	0·00	0·10	Day
2 p.m.	3·66	0·67	39·7	3·44	3·41	+0·03	3·42	5·58	1·63 / 0·50	0·50	11·15	,,	11·12	2·75	,,	0·27	Day
8 p.m.	2·73	1·22	38·2	3·55	3·60	−0·05	3·57	5·63	1·58 / 0·53	0·53	12·15	,,	5·73	1·42	,,	0·13	Day
2 a.m.	2·32	0·68	35·2	2·61	2·70	−0·09	2·65	4·63	1·75 / 0·75	0·75	12·10	0·00	7·46	1·85	0·00	0·62	Night

DISCUSSION OF RESULTS.

A. *The Relation between the Sugars and Starch of the Leaf.*

As in the mangold leaf during the early stages of growth, saccharose is the predominating sugar in the potato leaf—the curve of sac-

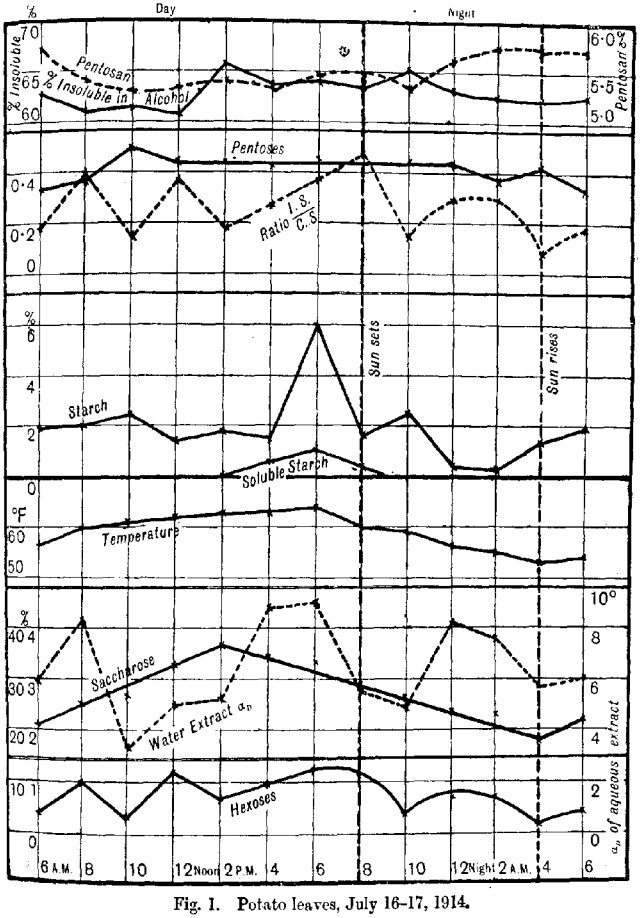

Fig. 1. Potato leaves, July 16–17, 1914.

charose in Fig. 1 is well above the curve of hexoses and is distinguished from it by its regularity. From 6 a.m. to 2 p.m. as the temperature

rises the saccharose increases along practically a straight line, which runs more or less closely parallel to the temperature curve. The maximum of saccharose is, however, reached earlier than the temperature maximum, viz. at 2 p.m.; after this the saccharose falls continuously along nearly a straight line, throughout the rest of the day and night, until sunrise next morning. The range of variation during the 24 hours is from 1·76 to 3·66 per cent.

The hexoses are present in relatively small amount and during the day fluctuate far more, and less regularly, than the cane sugar: the total variation is only from 0·4 to 1·2 per cent. The small changes in the hexoses between 6 a.m. and 2 p.m. synchronise with small changes in the starch present in the leaf, as if interconversion of these substances occurred. As will be seen later, if any reliance can be put upon the dextrose values, it is the dextrose which undergoes the greatest change (see Fig. 3). This sugar appears to fall from 8 a.m. to 10 a.m., whilst the starch increases; from 10 a.m. to noon the starch falls slightly and the dextrose increases. From 12 to 2 p.m. the starch increases and the dextrose falls almost to zero. After 2 p.m. the saccharose steadily falls whilst the hexoses increase, apparently owing to the inversion of the cane sugar, until 8 p.m. At the same time a sudden rise in the starch occurs between 4 p.m. and 6 p.m.; the starch which during the earlier part of the day had changed very little increases from 1·5 to 5·95 per cent. It is a striking fact that directly after the saccharose reaches its maximum at 2 p.m. the "soluble starch" (or dextrin) can be detected in the leaf material. This increases along a straight line until a maximum is reached at 6 p.m. which corresponds with the maximum of the starch. It is probable that the "soluble starch" is formed as an intermediate product between the hexoses (? dextrose) and the true, insoluble starch stored in the leaf. This form of starch is only to be detected in the leaf between 2 p.m. and about 9 p.m., its formation synchronising with the abnormally rapid increase of the starch, which occurs 2 or 3 hours before sunset. In this particular case, the starch stored in the leaf just before sunset is apparently very rapidly put under contribution again, as it falls in amount to about 1·6 per cent. at 8 p.m. The rapid fall of hexoses from 1·2 to 0·4 per cent. between 8 p.m. and 10 p.m. corresponds with a rise of starch from 1·6 to 2·6 per cent., whilst the fall of starch from 10 p.m. to midnight corresponds with a rise of hexoses. Between 12 midnight and 2 a.m. starch has almost disappeared from the leaf (0·2 to 0·3 per cent.), but just before sunrise, apparently in response to the first sign of daylight, the starch increases

to about 1·3 per cent., whilst the hexoses fall correspondingly. It is noteworthy that the starch appears to be formed in early morning considerably before the sugars show any increase. The slight increase of starch after sunset, between 8 p.m. and 10 p.m., at the expense of the hexoses is also very striking; at this time of day the intensity of the light was small.

Hexose-saccharose ratio. The curve showing the variation of this ratio naturally follows in its general outline the hexose curve with its abrupt changes. This is a consequence of the linear character of the saccharose curve.

Pentoses. These show a slight increase in the early part of the day, but from noon onwards are practically constant.

Pentosans and matter insoluble in alcohol. In the mangold leaf one of the most striking features was the absolute parallelism of the curves of pentosans and of matter insoluble in alcohol. This parallelism is almost entirely lost in the case of the potato leaf, apparently owing to the presence of starch and its precursors. At night, in particular, the pentosans appear to increase, whilst the matter insoluble in alcohol (including the starch) diminishes.

Rotation of the aqueous extract of the dried leaf tissue left after extracting the sugars. The curve showing the variation of the rotation of the aqueous extract of the dried leaf tissue from which all alcohol-soluble substances have been removed is probably an index of the variation of synthetical products intermediate between the hexoses and starch. Generally speaking this curve is intermediate in its character between the starch curve and the hexose curve. Table I shows that the rotation of this extract calculated as soluble starch points to the presence of considerable quantities of substances with a high *positive* rotation, which are possibly of the nature of gums but more probably are up-grade or down-grade products of starch, other than dextrin or soluble starch. They are generally not convertible into hexose by taka-diastase; it is only between 4 p.m. and 8 p.m. that a small quantity of a substance which is so convertible appears in the leaf. Even when this is present the rotation of the aqueous extract is from $2\frac{1}{2}$ to 4 times that corresponding with the "soluble starch" actually found.

During the early part of the day up to 12 noon the curve of the rotation, α_D, is more or less parallel with the hexose curve[1]; as the

[1] It must be borne in mind that the two curves (hexose and rotation curves) apply to different portions of the material analysed: the hexoses are estimated in the alcohol-soluble extract, whilst the rotation curve refers to the *aqueous* extract of the material left after all the substances soluble in alcohol have been removed.

hexoses rise so does the rotation of the aqueous extract and when the hexoses fall abruptly, as between 8 and 10 a.m., *when the starch increases*, the rotation also falls very greatly. From 10 a.m. to 2 p.m. the general character of the hexose and rotation curves is similar as regards rise and fall; from 2 p.m. onwards, when the cane sugar falls and the hexoses increase, there is a rapid rise of the rotation curve, which seems to follow more or less the formation of "soluble starch" and starch. The rotation curve reaches a maximum at the same time (6 p.m.) as the hexoses, soluble starch and true starch, and then falls abruptly, just as the starch curves fall, between 6 p.m. and 8 p.m. At night the rotation curve follows, on an exaggerated scale, the curve of hexoses and is the inverse of the starch curve.

The intimate relation existing between the three curves under discussion, which show the variation of the hexoses, starch and rotation of the aqueous extract of the sugar-free leaf, points to the starch and hexoses being readily interconvertible. The substance with high positive rotatory power which appears so intimately related to the starch and hexoses may either be an intermediate product in the synthesis of starch (other than dextrin or soluble starch) or a substance such as a protein or gum, with a high positive rotation, which stands in close causal relationship with this synthesis. In the present state of our knowledge it is useless to offer further conjectures.

B. *The Stalks and the Translocation of the Sugars.*

As in the mangold stalks, the saccharose remains practically constant in the potato stalk throughout the day (3·2 to 3·6 per cent.) in spite of a much larger variation of this sugar in the leaf (see Fig. 2). At night a slight fall occurs followed by an increase after sunrise to nearly the former level. The hexoses vary in somewhat the same way, but the range of variation is greater during the day (4·94 to 5·63) and the fall at night correspondingly larger (5·63 to 4·63). The curve of *apparent dextrose* (for data see Table V) is almost parallel to the saccharose curve and the same is true of the curve of *apparent laevulose*; the dextrose, as in the mangold stalk, always appears to be in large excess as compared with the laevulose, the ratio $\frac{D}{L}$ (pentoses as xylose) varying between 4·5 and 5·5. Although the laevulose and dextrose curves are practically parallel, the *absolute* increases during the day being nearly the same, the value $\frac{D}{L}$

falls considerably from 6 a.m. to 2 p.m., owing to the smallness of the laevulose values.

In the potato stalks, the fluctuations of the "apparent laevulose" are far less than in the mangold stalks (compare Fig. 2 with Figs. 7, 8 and 9 in preceding paper I), whilst the ratio of hexoses to saccharose remains nearly constant (see Table II) throughout the 24 hours. The variation of "apparent" dextrose and "apparent" laevulose is discussed later (see p. 373).

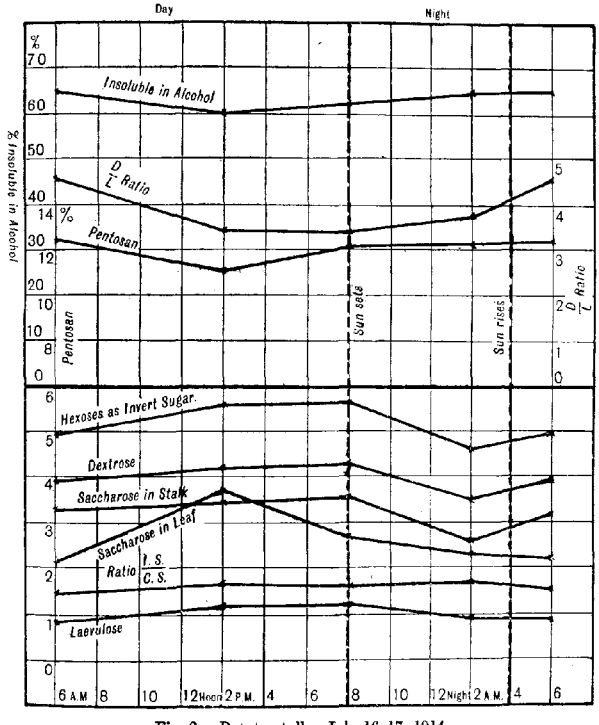

Fig. 2. Potato stalks, July 16–17, 1914.

The fact that in the leaf the saccharose is always greatly in excess of the hexoses (ratio $\frac{\text{I.S.}}{\text{C.S.}}$ varies from 0·08 to 0·45) whereas in the stalks the hexoses are always greatly in excess of the saccharose (ratio $\frac{\text{I.S.}}{\text{C.S.}}$

varies from 1·52 to 1·75) is best explained as in the case of the mangold by the view that in the leaf the saccharose is a primary product and is converted into hexoses for purposes of translocation.

Pentosans and Matter Insoluble in Alcohol.

In the stalks of the potato, unlike the leaves, practically no starch is present to interfere with the parallelism of the curves showing the pentosan content and the total leaf matter insoluble in alcohol (cellulose + lignified tissue) (see Fig. 2). From 6 a.m. to 2 p.m. the sugars and other substances soluble in alcohol are increasing so that the proportion of matter insoluble in alcohol falls. It should be noted that in the potato stalks the sugars form only a small proportion of the increase of total soluble matter; thus between 6 a.m. and 2 p.m. their increase is only 0·8 per cent., whilst the other soluble matters increase by 3·8 per cent.[1] After 2 p.m. the insoluble matter gradually increases to practically its earlier value, and the same is true of the pentosans.

The following table (Table III) gives a comparison between the potato and the mangold as regards the range of variation of the total sugars and of the matter soluble in alcohol in the leaf and stalk.

As regards the *leaf* constituents this table shows that the potato in its early stages of growth closely resembles the mangold at a corresponding stage; the range of variation of the sugars and of the substances soluble in alcohol is nearly the same in both cases. In both cases also the saccharose is greatly in excess of the hexoses. It is probable that, in the potato as in the mangold, during the later period of growth, when storage is the principal function, the relative proportion of saccharose and hexoses would be found to change, the hexoses then predominating in the leaf as well as in the stalks.

In the potato *stalks*, however, the actual proportion of substances soluble in alcohol is considerably less (35·2–39·7) than in the mangold (43·4–46·8), but the range of variation during the day is greater. The

[1] In the mangold stalks during the day the increase of the sugars is considerably greater than the increase of the total substances soluble in alcohol; during this period the soluble substances other than the sugars (amino-acids, tannins, amides) fall off greatly relatively to the sugars. Thus:

Mangold Stalks. Series I. August 26th–27th (average of top and bottom halves).
Increase of total sugars from 6 a.m. to noon = 4·87 %
Increase of total alcohol-soluble substances = 3·0 %

Mangold Stalks. Series II. September 10th–11th.
Increase of total sugars from 10 a.m. to 4 p.m. = 6·20 %
Increase of total alcohol-soluble substances = 1·5 %

variation of the sugars (1·93 per cent.) is however far less than in the mangold (4·68). The last two columns show how greatly the proportion of sugars and substances soluble in alcohol increases in the mangold in the later stages of growth.

TABLE III.

Range of Variation of Sugars and Alcohol-soluble Matter in the Mangold and Potato.

	Potato July 16–17, 1914	Mangold Series I, Aug. 26–27, 1913	Mangold Series II, Sept. 10–11, 1912	Mangold Series III, Oct. 11–12, 1912
Leaf:	%	%	%	%
Total sugars	1·91–4·93 Δ=3·02	1·70–5·27 Δ=3·57	9·62–17·17 Δ=7·55	14·5–21·0 Δ=6·5
Alcohol-soluble substances	34·1–39·3 Δ=5·2	37·2–42·5 Δ=5·3	44·2–54·7 Δ=10·5	47·9–54·95 Δ=7·05
Stalks:				
Total sugars	7·28–9·21 Δ=1·93	10·95–15·63 Δ=4·68	25·32–31·76 Δ=6·44	— —
Alcohol-soluble substances	35·2–39·7 Δ=4·5	43·4–46·8 Δ=3·4	64·2–66·9 Δ=2·7	— —

Table III shows that in both mangold and potato *leaves* the daily fluctuation of the substances soluble in alcohol is always far greater than (often nearly double) that of the total sugars. The same is true of the potato *stalk*, but in the mangold stalk the change in the sugars is always much greater than that of the alcohol-soluble constituents.

C. *The Dextrose-Laevulose Ratio.*

The "apparent" dextrose and laevulose have been calculated, as in the case of the mangold, on the assumption that the pentoses are either arabinose or xylose. The values are given in Tables IV and V.

D = percentage of apparent dextrose calculated on the total vacuum-dried matter (T.V.D.M.).

L = percentage of apparent laevulose calculated on the total vacuum-dried matter (T.V.D.M.).

I. *Leaves.*

As was the case in Series I of the mangold pickings (Paper II, p. 335) the results obtained for the "apparent" dextrose and laevulose are of little real value as an index of the true proportions of these sugars present, owing to the presence of optically active impurities which cannot be

removed by the ordinary treatment with basic lead acetate. The quantity of the reducing sugars is so small that the error introduced in this way becomes relatively very great; a large difference too is seen between the results for dextrose and laevulose according as the pentose is assumed to be arabinose or xylose. This is not because the pentose is actually present in large amount (it ranges only from 0·35 to 0·52

TABLE IV.

Apparent Dextrose and Laevulose in Potato Leaves.
July 16th–17th, 1914.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %		Hexoses % calc. as invert sugar	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Pentose as arabinose	Pentose as xylose		
6 a.m.	Nil	0·42	0·00	Nil	0·42	0·00	0·42	0·42	0·40	
8 a.m.	0·26	0·76	0·34	0·46	0·53	0·85	1·01	1·00	1·00	Day
10 a.m.	Nil	0·38	0·00	Nil	0·38	0·00	0·38	0·38	0·37	
12 noon	0·25	0·95	0·26	0·48	0·73	0·66	1·23	1·21	1·21	
2 p.m.	Nil	0·69	0·00	Nil	0·69	0·00	0·69	0·69	0·67	
4 p.m.	Nil	0·97	0·00	Nil	0·97	0·00	0·97	0·97	0·93	
6 p.m.	0·19	1·11	0·17	0·45	0·83	0·54	1·30	1·28	1·27	
8 p.m.	0·17	1·08	0·16	0·39	0·83	0·47	1·25	1·22	1·22	
10 p.m.	Nil	0·42	0·00	Nil	0·42	0·00	0·42	0·42	0·40	Night
12 night	Nil	0·76	0·00	0·18	0·57	0·32	0·76	0·75	0·73	
2 a.m.	Nil	0·71	0·00	0·19	0·50	0·38	0·71	0·69	0·68	
4 a.m.	0·01	0·15	0·06	0·15	Nil	∞	0·16	0·15	0·15	

TABLE V.

Apparent Dextrose and Laevulose in Potato Stalks.
July 16th–17th, 1914.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %		Hexoses % calc. as invert sugar	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Pentose as arabinose	Pentose as xylose		
6 a.m.	3·68	1·11	3·32	3·91	0·86	4·58	4·79	4·77	4·94	Day
2 p.m.	3·90	1·53	2·55	4·17	1·23	3·39	5·43	5·40	5·40	
8 p.m.	3·95	1·55	2·55	4·23	1·24	3·41	5·50	5·47	5·63	
2 a.m.	3·25	1·25	2·60	3·53	0·94	3·75	4·50	4·47	4·63	Night

per cent. on the T.V.D.M.), but because the quantity and rotatory power of the hexoses is exceedingly small (0·15 to 1·27 per cent.). At 8 a.m., for instance, if the pentoses are taken as arabinose, $\frac{D}{L} = 0{\cdot}34$, but if they are assumed to be xylose $\frac{D}{L}$ becomes 0·85.

That the results are vitiated by the presence of a *laevo*-rotatory impurity appears clearly in the data for 6 a.m., 10 a.m., 2 p.m., 4 p.m. and 10 p.m., in all of which cases the amount of dextrose appears to be *nil*. If in these cases the whole of the reducing sugar is assumed to be laevulose, the negative rotation calculated does not account, on the assumption that the pentose is xylose, for the negative rotation actually observed.. The following table shows the differences:

Time	Actually observed for hexoses in 200 mm. tube at 20°*	Calculated for hexose = laevulose	Rotation not accounted for
6 a.m.	−0·143°	−0·060°	−0·083°
10 a.m.	−0·076	−0·036	−0·040
2 p.m.	−0·075	−0·055	−0·020
4 p.m.	−0·146	−0·102	−0·044
10 p.m.	−0·063	−0·039	−0·024

* After allowing for the pentoses (as xylose) and saccharose present.

If the results are calculated on the assumption that the pentose is arabinose, the negative rotation not accounted for becomes even greater; thus at 6 a.m. it becomes − 0·138° instead of − 0·083°. The number of cases in which dextrose appears to be entirely absent is increased on this assumption.

It is clear therefore that the apparent predominance of laevulose in the potato *leaf* is due to the presence of relatively large quantities of a laevo-rotatory impurity (? asparagine), and it is probable that the dextrose and laevulose, as in the mangold leaf, are really present in equal proportions, that is as invert sugar, and are formed from saccharose. It is interesting that the dextrose appears in largest amount at 6 p.m. and 8 p.m., that is at the time when the starch content reaches a maximum and is subsequently put under contribution. As we show on p. 357, the starch is broken down by the leaf enzymes completely to dextrose. Fig. 3 shows the variation of the apparent dextrose and laevulose during the 24 hours (pentoses assumed to be xylose); as an index of the real fluctuation of the hexoses the curves have, of course, no value, but they are interesting as showing that the laevo-rotatory impurity varies regularly during the 24 hours. The laevulose always

appears in considerable excess except just about sunrise (4 a.m.) when the whole of the very small amount of hexose present (0·15 per cent.) appears as *dextrose* not laevulose, and a *positive* rotation of + 0·015 remains unaccounted for. A somewhat similar abnormality was found just before sunrise in the case of the mangold leaves, Series I; whereas during the greater part of the 24 hours *dextrose* appeared to be in excess (see Table I, Paper II, p. 332, arabinose values) in the mangold leaf, at 4 a.m., when the total hexose was exceedingly small (0·2 per cent.), laevulose suddenly appeared to predominate; at the same time, the

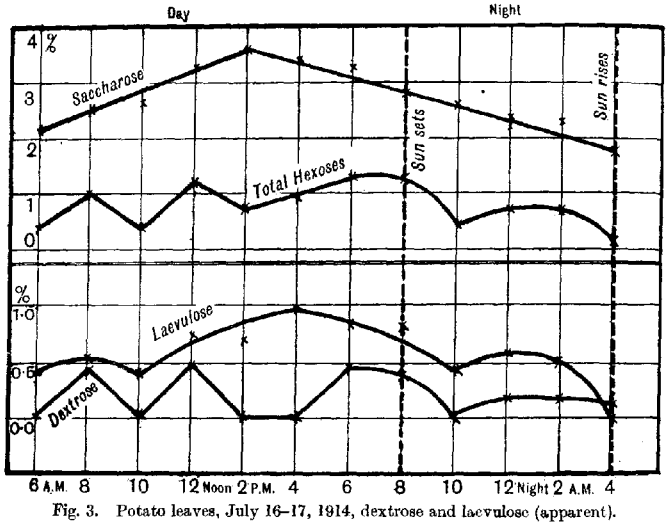

Fig. 3. Potato leaves, July 16–17, 1914, dextrose and laevulose (apparent). (Pentose as xylose.)

polarisation results for cane sugar, which in general throughout the day were *higher* than the reduction values, suddenly became *lower*. But 2 hours later, at 6 a.m., the values were again much higher. The following data illustrate this:

Mangold Leaves. Series I. August 26th–27th, 1913.

	D %	L %	$\frac{D}{L}$	Δ* in saccharose values
2 a.m. ...	0·29	0·08	3·62	+ 7·3
4 a.m. ...	0·00	0·20	0	−29·2
6 a.m. ...	0·73	0·00	∞	+42·6

Sunrise = 5.5 a.m

* Δ = difference between values found for saccharose by polarisation and by reduction.

It would appear, therefore, that in the potato as in the mangold leaf *two* oppositely active impurities are present at different times of the day. During the greater part of the day laevulose appears to be in excess owing to a laevo-rotatory impurity predominating, but *at night* the amount of this impurity diminishes until it is replaced just before sunrise (4 a.m.) by an excess of *dextro*-impurity. The variation is well seen by considering the data obtained by assuming the pentoses to be arabinose; the amount of laevo-rotation left unaccounted for when the whole of the hexose is assumed to be laevulose gradually drops from 10 p.m. to 2 a.m., whilst at 4 a.m. dextrose appears to be present.

At 10 p.m. negative rotation unaccounted for = −0·062° (200 mm. tube)
12 midnight negative rotation unaccounted for = −0·010°
2 a.m. negative rotation unaccounted for = −0·002°
4 a.m. positive rotation unaccounted for = +0·015°

Between 6 a.m. and 8 a.m., that is just after sunrise, the quantity of *negative* impurity suddenly increases very largely, the negative reading unaccounted for at 6 a.m. being greater than at any other period of the 24 hours (− 0·138° if pentose is arabinose, − 0·083° if xylose).

The following table (Table VI) shows how the presence of the optically active impurities causes abnormally large differences in the results found for saccharose by the reduction and by the polarisation methods. This table should be compared with the similar table obtained in the case of the mangold leaf (see p. 338, preceding paper).

TABLE VI.

Divergence of Results for Saccharose by the Reduction and Polarisation Methods—Potato Leaves. July 16th–17th, 1914.

	Citric acid inversion			Invertase inversion			$\frac{D}{L}$
Time	% saccharose reduction	% saccharose polarisation	Δ%	% saccharose reduction	% saccharose polarisation	Δ%	(pentose xylose)
6 a.m.	2·16	2·48	+14·8	2·11	2·70	+17·1	0·00
8 a.m.	2·47	2·37	− 4·0	2·60	2·67	+ 2·7	0·85
10 a.m.	2·81	2·71	− 3·5	2·65	3·10	+17·0	0·00
12 noon	3·39	3·63	+ 7·1	3·19	3·41	+ 6·9	0·66
2 p.m.	3·81	4·46	+17·0	3·50	4·54	+29·7	0·00
4 p.m.	3·56	3·75	+ 5·3	3·34	3·83	+14·6	0·00
6 p.m.	3·46	4·34	+25·4	3·22	4·48	+39·2	0·54
8 p.m.	2·77	2·21	−20·2	2·69	2·98	+10·8	0·47
10 p.m.	2·76	2·31	−16·2	2·49	2·77	+11·2	0·00
12 night	2·48	2·46	− 0·8	2·30	2·39	+ 3·9	0·32
2 a.m.	2·38	1·91	−19·8	2·26	1·98	−12·4	0·38
4 a.m.	2·09	2·43	+16·2	1·44	2·51	+74·4	∞

As in the case of the mangold leaves, the polarisation results for saccharose are generally considerably *higher*—often 20 to 30 per cent. higher—than the reduction values[1]. The two methods only give approximately the same results when $\frac{D}{L}$ approximates to 1 (e.g. at 8 a.m. and 12 noon, when Δ (invertase) is only 2·7 and 6·9 per cent.). When the dextrose appears to disappear $\left(\frac{D}{L}=0\right)$ the divergences (Δ) are greatest (e.g. 6 a.m., 10 a.m., 2 p.m., 4 p.m., 10 p.m.). As showing the presence of *two* distinct and oppositely active impurities, it is interesting that between 6 a.m. and 4 p.m. when the laevulose on the whole appears to increase (see Fig. 3) a rise in the apparent dextrose corresponds with a diminution in the difference between polarisation and reduction values for saccharose, and *vice versa*; but from 4 p.m. to 10 p.m. (laevulose falling) an increase in the apparent dextrose carries with it an increase in the divergence, whilst a fall in the dextrose is accompanied by an opposite result (compare the figures in Table VI with the curves in Fig. 3). Between 10 p.m. and 2 a.m., when the laevulose again appears to rise and fall, the difference between the two sets of values becomes less and less (invertase figures) and finally *negative*. It is interesting to compare the following figures:

				Sunrise	
	10 p.m.	Midnight	2 a.m.	4 a.m.	6 a.m
Rotation not accounted for by the hexoses ...	−0·062°	−0·010°	−0·002°	+0·015°	−0·081°
Δ (invertase) between saccharose values ...	+11·0 %	+ 3·8 %	−12·5 %	+74 %	+17·2 %

The abrupt change between 2 a.m. and 4 a.m. (corresponding with the sudden fall to zero of the apparent laevulose) from a negative rotation not accounted for to a high positive value, and a negative difference −12·5 per cent. to a high positive value +74 per cent., is followed, *immediately after sunrise*, by equally great changes in the

[1] If the discrepancy between the results for saccharose were due solely to an amino-acid or amide such as asparagine, one would expect the divergence to be diminished by taking the first (direct) polarisation after saturating the solution with sulphur dioxide so as to make it strongly acid (see Pellet, *Dosage du Sucre par Inversion*, 1913). As a matter of fact in the case of the potato whether the direct reading was taken (as has been usual in our experiments) in practically neutral solution or whether it was taken in acid solution (SO_2) made very little difference in the majority of cases, the figures usually being very high as compared with the reduction values.

opposite direction. The fluctuations, whatever be their cause, show throughout evidences of periodicity; this appears most clearly in the shape of the curve of apparent laevulose.

II. *Stalks.*

The results for the potato stalks closely resemble those found for the mangold stalks in the fact that the dextrose present appears always to be in large excess as compared with the laevulose; the ratio $\frac{D}{L}$ varies from 3·39 to 4·58 (pentose as xylose). But there is this striking difference: in the mangold, dextrose appeared to be the predominant sugar in both leaf and stalks, but in the potato it is in excess *only in the stalks*, whilst in the *leaf*, as pointed out above, laevulose predominates. It is also very striking, that whereas in the mangold the greatest fluctuations and the greatest divergences between the reduction and polarisation values for saccharose were found in the stalks and mid-ribs (Δ varied from + 40 per cent. to − 90 per cent., see Table VIII, preceding paper), caused no doubt by large fluctuations in the optically active impurities present, in the potato stalks *the differences are as a rule relatively small, and, in general, less than in the leaves.*

The following table (Table VII) shows this:

Table VII.

Divergence of Values of Saccharose by Polarisation and Reduction Methods—Potato Stalks. July 16th–17th, 1914.

	Citric acid inversion			Invertase inversion			
Time	Saccharose by reduction	Saccharose by polarisation	Δ %	Saccharose by reduction	Saccharose by polarisation	Δ %	$\frac{D}{L}$ (xylose
	%	%		%	%		
6 a.m.	3·20	3·70	+15·6	3·28	3·62	+10·4	4·58
2 p.m.	3·44	3·84	+11·6	3·41	3·92	+14·9	3·39
8 p.m.	3·55	3·35	− 5·6	3·60	3·85	+ 7·0	3·41
2 a.m.	2·61	2·82	+ 8·0	2·70	2·88	+ 6·7	3·75

The extreme divergence here is only 15 per cent., whilst in general the divergence (Δ) is less than 10 per cent. There are no such abrupt changes from positive values to negative values as were met with in the mangold stalks (see p. 344) and had their counterpart in the sudden variation in the values for apparent laevulose (see Figs. 7 and 8, Paper I). One of the most striking differences between the potato stalks and the

mangold stalks is that in the former the curves *of apparent dextrose and apparent laevulose run almost parallel to one another* throughout the 24 hours, and at the same time about parallel to the saccharose curve (see Fig. 2, p. 371). Each sugar increases slightly and continuously during the day and then falls at night.

It seems probable from the parallelism of the curves of saccharose and total hexoses that the dextrose and laevulose are actually present in the stalks as invert sugar, being formed from the saccharose by inversion; the large apparent excess of dextrose would then be due to the presence of a *dextro*-rotatory impurity which accumulates in the stalks (whereas in the leaf a *laevo*-rotatory substance is generally in excess). The divergence Δ between the reduction and polarisation values is relatively small in the case of the potato because the substance is of such a nature that the *change* of rotation brought about by the processes of inversion is relatively small; but the existence of this divergence (up to 15 per cent.) is a proof that some such compound is present. On the other hand the practical parallelism of the curves of apparent dextrose and laevulose, which is in striking contrast with the *mangold* stalks, suggests that whatever be the impurity which is present, its amount remains relatively constant throughout the 24 hours.

On the other hand the relatively small divergence between the polarisation and reduction values for saccharose in the potato stalks, in contrast with the large divergences found in the mangold stalks, may be taken to mean that only small amounts of the optically active impurity are present in the potato stalks and that the values of dextrose and laevulose in the stalks (*not* in the leaves) nearly represent the true values for these sugars. If this is the case the dextrose has accumulated in the stalks far more than the laevulose, possibly owing to the latter sugar being used up for tissue building[1], and to the fact that the starch formed in the leaf gives dextrose as sole product when hydrolysed by the leaf enzymes (see p. 357). One would naturally expect the starch in the tuber to be built up from dextrose as it yields dextrose exclusively when hydrolysed by either acids or taka-diastase and the predominance of dextrose in the stalks conveying sugars to the tuber would be quite natural if this were the case. The question, however, whether the dextrose is in actual excess over the laevulose in the stalks or whether

[1] It is interesting to recall Meyer's observation in 1886 that almost all leaves capable of forming starch at all produce it abundantly from a 10 per cent. solution of laevulose and a relatively small number only from dextrose. On general grounds, considering the relationship of starch and dextrose, one would have expected the reverse to be the case.

the two sugars are present mainly in the form of invert sugar can only be decided definitely when methods have been devised of estimating the two sugars, in presence of each other, which are free from the errors caused by optically active impurities.

Summary.

1. In the potato leaf when the tubers are beginning to develop the principal sugar present is saccharose; its amount increases from sunrise up to 2 p.m., following approximately the curve of temperature. It then falls during the rest of the day and night. The rise and fall are both linear.

2. The hexoses are present in the leaf in very small amounts—generally less than 1 per cent. of the total dry weight of the leaf. They fluctuate considerably during the early part of the day, the fluctuations being apparently determined by conversion into or formation from starch.

3. During the early part of the day up to 2 p.m. the proportion of starch changes very little, the small fluctuations which occur being related to changes in the starch. The starch is apparently formed from the hexoses.

4. Directly the amount of saccharose has reached its maximum at 2 p.m. the hexoses begin to increase in the leaf, owing apparently to hydrolysis of the saccharose to invert sugar; at the same time "soluble starch" (or dextrin) is first detected in the leaf and its amount increases regularly up to 6 p.m. At 6 p.m., 2 hours before sunset, the true starch in the leaf reaches a maximum value, far greater than any previous value during the day. The starch and "soluble starch" subsequently fall rapidly until between midnight and 2 a.m. the amount left is exceedingly small (0·2 per cent.). The starch is apparently converted directly into hexose (dextrose), the amount of which increases in the leaf.

5. In the stalks reducing sugars predominate greatly over the saccharose in spite of the fact that in the leaf the latter is in excess. As in the mangold it is probable that cane sugar is the first sugar formed in the leaf and that it is hydrolysed by invertase in the veins, mid-ribs and stalks, for the purpose of translocation.

6. As in the mangold, the true proportions of dextrose and laevulose cannot be determined in the leaves and stalks owing to the presence of soluble optically-active impurities, which vitiate the polarimetric data.

It is shown that the presence of these impurities also falsifies the results obtained for saccharose by the double polarisation method. The fluctuations of the "apparent dextrose" and "apparent laevulose" in the leaf really indicate variations in these impurities rather than variations in the hexoses, which are perhaps present mainly as invert sugar. In the stalks, where the amount of optically active impurity appears to be less than in the leaves, it is possible that the dextrose is actually in excess as it appears to be, and that the starch in the tuber is built up from this sugar.

7. Maltose is invariably absent from the potato leaf and also from the leaves of other plants which form much starch in the leaf. The degradation of starch in the leaves is probably effected by a mixture of enzymes similar to the enzymes of *Aspergillus oryzae* (taka-diastase); maltase is always in relative excess, so that the starch is degraded completely to dextrose. The series of changes is therefore:

Starch → dextrins → maltose → dextrose.

Appendix. Experimental Data.

Potato Leaves. July 16th–17th, 1914.

In the first two analyses (6 a.m. and 8 a.m.), after the treatment with basic lead acetate, the precipitate was washed to 2 litres, but the first and second litre of washings were analysed separately so as to obtain an idea of the amount of sugars left behind when the washing was continued only to 1 litre. As it was found that this was quite appreciable, in all the later analyses the lead precipitate was washed to nearly 2 litres, and after precipitation with sodium carbonate the solution was made to 2000 cc. (= A).

The extract of the leaf material was evaporated *in vacuo* and made up to 500 cc. 440 cc. of the 500 were treated with basic lead acetate and the precipitate washed to 1 litre; the filtrate was treated with solid sodium carbonate and made to 1000 cc. = A.

The second litre of washings was also treated with solid sodium carbonate and made up to 1000 cc. = A'.

Time	Vacuum-dried matter soluble in alcohol, grms.	Vacuum-dried matter insoluble in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution A used for reduction (x)	Polarisation of A in 200 mm. tube*, $\alpha_D^{20°}$	Reduction of x cc. of solution A, grms. CuO	Invertase inversion: Reduction of x cc. after inversion, grms. CuO	Invertase inversion: α_D after inversion (200 mm.)	Citric inversion: Reduction of x cc. after inversion, grms. CuO	Citric inversion: α_D after inversion (200 mm.)	Phloroglucide from 50 cc. A	Remarks
6 a.m.	34·32	57·91	92·23	25 cc. A	+0·115°	0·0426	0·1445	−0·104°	0·1470	−0·108°	0·0169	1st litre (A)
	,,	,,	,,	25 cc. A'	±0·000	0·0032	—	—	0·0125	−0·000	—	2nd litre (A')
8 a.m.	43·30	67·55	110·85	25 cc. A	+0·261	0·0835	0·2300	−0·075	0·2223	−0·050	0·0127	1st litre (A)
	,,	,,	,,	25 cc. A'	−0·002	0·0024	—	—	0·0190	−0·022	—	2nd litre (A')
10 a.m.	44·15	70·58	114·73	25 cc. A	+0·117	0·0387	0·1247	−0·078	0·1298	−0·061	0·0077	A = 2000 cc.
12 noon	44·75	69·24	113·99	25 cc. A	+0·183	0·0513	0·1548	−0·056	0·1612	−0·066	0·0054	,,
2 p.m.	34·57	66·72	101·29	25 cc. A	+0·149	0·0312	0·1318	−0·051	0·1408	−0·050	0·0045	,,
4 p.m.	49·38	87·17	136·55	25 cc. A	+0·139	0·0505	0·1800	−0·096	0·1887	−0·100	0·0071	,,
6 p.m.	43·22	77·50	120·72	25 cc. A	+0·189	0·0578	0·1684	−0·107	0·1767	−0·101	0·0069	,,
8 p.m.	59·25	102·42	161·67	25 cc. A	+0·190	0·0725	0·1960	−0·090	0·1998	−0·042	0·0094	,,
10 p.m.	39·74	74·30	114·04	25 cc. A	+0·120	0·0272	0·1073	−0·061	0·1161	−0·041	0·0059	,,
Midnight	43·87	76·07	119·94	25 cc. A	+0·133	0·0390	0·1168	−0·044	0·1232	−0·047	0·0063	,,
2 a.m.	53·22	88·90	142·12	25 cc. A	+0·168	0·0415	0·1328	−0·024	0·1375	−0·020	0·0063	,,
4 a.m.	42·58	70·36	112·94	25 cc. A	+0·147	0·0188	0·0648	−0·035	0·0857	−0·032	0·0053	,,

* In most cases the solution was sufficiently colourless to allow the reading to be taken in a 400 mm. tube. The data given are, however, all reduced to the 200 mm. standard

Potato Stalks. July 16th–17th, 1914.

Distilled *in vacuo* and made up to 250 cc. 190 cc. of the 250 treated with basic lead acetate, filtered and washed to 1 litre, solid sodium carbonate added and made up to 1000 cc. = Solution A.

Time	Vacuum-dried matter soluble in alcohol, grms.	Vacuum-dried matter insoluble in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution A used for reduction (x)	Polarisation of A in 200 mm. tube, $\alpha_D^{20°}$	Reduction of x cc. of solution A, grms. CuO	Invertase inversion: Reduction of x cc. after inversion, grms. CuO	Invertase inversion: α_D after inversion (200 mm.)	Citric inversion: Reduction of x cc. after inversion, grms. CuO	Citric inversion: α_D after inversion (200 mm.)	Phloroglucide from 50 cc. A
6 a.m.	31·47	56·24	87·71	25 cc. A	+0·468°	0·2200	0·3563	+0·023°	0·3525	+0·019°	0·0090
2 p.m.	29·27	44·45	73·72	25 cc. A	+0·385	0·2094	0·3292	+0·001	0·3304	+0·005	0·0085
8 p.m.	29·79	48·14	77·93	25 cc. A	+0·422	0·2242	0·3568	+0·012	0·3550	+0·038	0·0102
2 a.m.*	25·10	46·14	71·24	25 cc. A	+0·345	0·1914	0·2950	+0·021	0·2918	+0·023	0·0170

* In this analysis the extract was made to 200 cc. (not 250) and 170 cc. of the 200 treated with basic lead acetate.

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